Department of Pathology and Laboratory Medicine

Annual Research Symposium

Highlighting the Research Conducted by Our Predoctoral Students, Residents, and Postdoctoral/Clinical Fellows

September 17, 2020

The Brinkhous-Bullitt Building

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Thursday, September 17, 2020

Schedule of Events

Introduction, Oral Presentations, and the Virtual Happy Hour Hosted via Zoom Using this Link:

https://zoom.us/j/93224048732?pwd=Wi9WbGV6OTdWeEYyZml5NHB2SVE3dz09

Meeting ID: 932 2404 8732 Passcode: 423242

Poster Previewing via this Link (available prior to, and the day of the symposium):

https://sakai.unc.edu/portal/site/dplm_2020_symposium (You may need to cut and paste this address into your browser. You will need to sign in to Sakai using your ONYEN and password)

Live Discussion with Poster Presenters during Poster Sessions via the Zoom Links on the Lists of Posters (pages 10-11, and 22) or on the Sakai Site

SCHEDULE

9:55 **Introduction and Welcoming Remarks** (see Zoom link above)
Russell Broaddus, M.D., Ph.D., *Joe W. and Evelyn M. Grisham Distinguished Professor and Chairman, Pathology and Laboratory Medicine*

10:00-11:00 **Oral Presentations I** (see Zoom link above)
Moderator: Alina Hamilton

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Abigail Shelton

Thrombin-PAR1 Signaling in Pancreatic Cancer Promotes an Immunosuppressive Microenvironment
Yi Yang

Characterization of the Involvement of NF-κB Signaling in DAB2IP-Deficient ER+ Breast Cancer
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11:00-11:30 **Poster Preview Session I (**See Sakai Poster Previewing link above**)**

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12:15-1:00 **Lunch Break**

1:00-2:00 **Oral Presentations II** (see Zoom link above)
Moderator: Angana Mukherjee

Activation of Endogenous Transcription Factors by CRISPRa Mediates Cardiac Reprogramming
Benjamin Keepers

Functional Evaluation of CYLD in HPV+ Head and Neck Squamous Cell Carcinoma
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Genotype-Kinome Guided Development of Precision EGFR-Targeted Therapeutics for Glioblastoma
Erin Smithberger

Plasminogen Deficiency Mitigates Liver Damage Associated with Diet-Induced Obesity, but Does Not Alter Weight Gain
Woosuk Hur

2:00-2:30 **Poster Preview Session II (**See Sakai Poster Previewing link above**)**

2:30-3:15 **Poster Session II (**Use the Zoom links found in the Lists of Posters Presentations, pgs 10-11 and 22, or on the Sakai site**)**

4:30-5:30 **Virtual Happy Hour, including:** (see Zoom link above)
Best Presentation Awards
Trainee Choice Award
Joe W. Grisham Award for Excellence in Graduate Student Teaching

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Graduate Student, Resident, Postdoctoral Fellow, and Clinical Fellow Speaker Abstracts

Acquired Resistance to Targeted Inhibitors in EGFR-Driven Glioblastoma: Identification of Dual Kinase Targets

Abigail Shelton[1,4], Erin Smithberger[1,4], Madison Butler[1], Allie Stamper[6], Ryan E. Bash[1,4], Steven P. Angus[5], Mike East[2], Gary L. Johnson[2,3], Michael E. Berens[6], Frank B. Furnari[7], and C. Ryan Miller [1,4]

[1]Pathology & Laboratory Medicine, [2]Pharmacology, [3]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC, [4]University of Alabama Birmingham, Birmingham, AL, [5]Department of Pediatrics, Indiana University, Indianapolis, IN, [6]Department of Cancer and Cell Biology, Translational Genomics Research Institute, Phoenix, AZ, [7]Department of Pathology, University of California San Diego, San Diego, CA

Glioblastoma (GBM) is a devastating primary brain tumor with 5-year survival <5%. *CDKN2A* deletion (~60%) and *EGFR* amplification (55-60%) mutations frequently co-occur in GBM. Several EGFR tyrosine kinase inhibitors (TKI) have failed clinically, due in part to acquired resistance. To mechanistically examine this type of resistance, we used genetically engineered mouse astrocytes harboring *Cdkn2a* deletion and EGFRvIII, a common (~35%) activating mutation. Resistant cells were generated via chronic exposure to gefitinib or erlotinib. Resistance to these first-generation EGFR TKI conferred cross-resistance (up to 36-fold ΔIC_{50}) to a panel of second and third generation TKI relative to sensitive parental lines. Moreover, integrated RNA sequencing (RNA-seq) and chemical proteomics (multiplexed inhibitor beads and mass spectrometry (MIB-MS)) showed that the kinase transcriptome and proteome were rewired in resistant cells: 113 and 159 of ~300 detected kinases were differentially expressed (DE, $p<0.05$), respectively. We then examined acute (≤48h) kinome changes in both sensitive and resistant cells upon treatment with EGFR TKI afatinib. While treatment-naïve, sensitive cells achieved maximal kinome response at 24h (23% DE kinases), resistant lines had more DE kinases at 48h than 24h (19% and 12%, respectively), likely due in part to EGFR TKI cross-resistance. Overall, upregulated kinases include those implicated in the biology of gliomas (Bmx, Fgfr2) and other cancers (Pdgfrb, Mapk3/4, Ddr1/2, Pdk2). These kinases represent putative druggable targets for dual inhibition therapy. Integrated kinome profiling in GBM models with defined mutational profiles provides a powerful framework to identify novel therapeutic targets that could significantly alter current treatment paradigms.

Thrombin-PAR1 Signaling in Pancreatic Cancer Promotes an Immunosuppressive Microenvironment

Yi Yang[1], Patrick G. Schweickert[2], Emily E. White[2], Gregory M. Cresswell[3], Bennett D. Elzey[3], Timothy L. Ratliff[3], Paritha Arumugam[4], Stephen F. Konieczny[2], Matthew J. Flick[1]

[1]Department of Pathology and Laboratory Medicine, Lineberger Comprehensive Cancer Center and Blood Research Center, University of North Carolina School of Medicine, Chapel Hill, NC, [2]Department of Biological Sciences and the Purdue Center for Cancer Research, Purdue University, West Lafayette, IN, [3]Department of Comparative Pathobiology and the Purdue Center for Cancer Research, Purdue University, West Lafayette, IN, [4]Cincinnati Children's Hospital Medical Center, Division of Pulmonary Biology, Cincinnati, OH

Background: Pancreatic ductal adenocarcinoma (PDAC) is characterized by a prothrombotic state and a poor anti-tumor immune responsiveness. Linking these two key features, we demonstrated thrombin-protease-activated receptor-1 (PAR1) signaling drives tumor growth by evading cytotoxic $CD8a^+$ cells. We now seek to identify key PAR1 downstream targets that promote PDAC tumor immune evasion.**Methods:** Syngeneic mixed cell subcutaneous tumor growth, unbiased transcriptional analyses, and functional tests of immunosuppressive response genes were employed to identify cellular and molecular immune evasion mechanisms mediated by thrombin-PAR-1 signaling in mouse PDAC tumor cells. **Results:** Elimination of tumor cell PAR1 in syngeneic graft studies increased cytotoxic T lymphocyte (CTL) infiltration and decreased tumor-associated macrophages in the tumor microenvironment. Co-injection of PAR1-expressing and PAR1-knockout ($PAR\text{-}1^{KO}$) tumor cells into immunocompetent mice resulted in preferential elimination of $PAR\text{-}1^{KO}$ cells from developing tumors, suggesting that PAR1-dependent immune evasion is not reliant on CTL exclusion. Transcriptomics analyses revealed no PAR1-dependent changes in the gene expression of immune checkpoint proteins and no difference in MHC-I expression. Importantly, thrombin-PAR1 signaling in PDAC cells upregulated genes linked to immunosuppression, including *Csf2* and *Ptgs2*. Functional analyses confirmed that both *Csf2* and *Ptgs2* are critical for PDAC tumor growth and overexpression of each factor partially restored tumor growth of $PAR1^{KO}$ cells in immunocompetent mice. **Conclusions:** Our results provide novel insights into the mechanisms of a previously unrecognized pathway coupling coagulation to PDAC immune evasion by identifying PAR1-dependent changes in the tumor microenvironment, a PAR1-driven immunosuppressive gene signature, and *Csf2* and *Ptgs2* as critical PAR1 downstream targets.

Characterization of the Involvement of NF-κB Signaling in DAB2IP-Deficient ER+ Breast Cancer

Angana Mukherjee[1], Daniel Hollern[2], Audrey Chang[2], Xianlu Peng[2], Albert Baldwin[1,2]

[1]Department of Pathology and Laboratory Medicine, [2]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC

Transcriptional profiling has classified breast cancer into different subtypes. Importantly, the majority of breast cancers are ER+ luminal tumors that exhibit overall good outcome due to ER-targeting therapies, but a significant subset is associated with frequent metastatic recurrence and resistance to endocrine therapy. Studies have suggested that loss of two RasGAP tumor suppressors - DAB2IP and RASAL2 - promotes poor outcome in luminal B breast tumors by activating Ras and NF-κB signaling. Nevertheless, the relationship between NF-κB pathway and RasGAPs in the luminal A subtype

remains unexplored. This study investigates the effect of loss of RasGAPs on NF-κB pathway in ER+ luminal A tumors. Our data has identified an NF-κB gene signature regulated by loss of DAB2IP in ER+ breast cancers. DESeq and Kaplan-Meier analysis were performed to mine the expression profiles of breast cancer patients from the TCGA database. The data indicate that low DAB2IP expression is associated with a significant decrease in relapse-free survival in patients with luminal A tumors. We have identified significant differential gene expression with loss of DAB2IP in ER+ malignancies, and observed that: 1) NF-κB target genes and effectors exhibit altered expression in these tumors, 2) DAB2IP loss upregulates unique sets of NF-κB regulated genes that may play a role in endocrine therapy resistance. Further we are investigating a gene panel for distinguishing between low and high DAB2IP breast cancers. We hypothesize that this gene signature will suggest mechanisms underlying the aggressiveness and therapy resistance of ER+ luminal breast cancers with loss of DAB2IP.

Development of a Model to Understand the Role of the Plasminogen Activation System in Pancreatic Tumor-Associated Venous Thrombosis

Keely G. Davey, Yi Yang, Zhaoming Tang, Matthew J. Flick, and Alisa S. Wolberg
Department of Pathology and Laboratory Medicine and UNC Blood Research Center, University of North Carolina School of Medicine, Chapel Hill, NC

Pancreatic ductal adenocarcinoma (PDAC) has the highest risk of cancer-associated thrombosis. The prothrombotic potential of PDAC has been linked to high expression of tissue factor (TF). TF drives thrombin activity in the tumor microenvironment (TME) and activates tumor cell protease activated receptor-1 (PAR-1). Preliminary data suggest that PAR-1 signaling initiates the plasminogen activation (PA) system which plays a critical role in PDAC tumor progression and thrombotic potential. We seek to determine how the PA system within the TME influences the prothrombotic properties of PDAC tumor cells by combining orthotopic injection of PDAC tumor cells with a murine model of venous thrombosis. KPC2 tumor cells, generated from KPC2 ($Kras^{G12D/+}$, $p53^{R172H/+}$, $Elas^{CreER/+}$) mouse tumors, were altered using CRISPR technology to prepare TF, urokinase (uPA), and urokinase receptor (uPAR) knockout (KO) lines. Cell lines were evaluated using thrombin generation and fibrin formation assays. Orthotopic injections were performed on 6-10-week old male C57Bl/6J mice and IVC stasis was performed 3 - 10 days thereafter. In vitro, uPA KO and uPAR KO cell lines exhibited the same procoagulant properties as unaltered KPC2 cells while TF KO cells had reduced procoagulant activity. Growth of KPC2 tumors in mice was not observed until 8 days, consistent with previous studies. Following IVC stasis, tumor-bearing mice had reduced survival compared to control mice. Identification of the specific mechanisms mediating PA-dependent PDAC tumor progression, invasion, and metastasis may reveal strategies to suppress PDAC progression and reduce the frequency and risk of PDAC-driven thrombosis.

Activation of Endogenous Transcription Factors by CRISPRa Mediates Cardiac Reprogramming

Benjamin Keepers[1,2], Sam Shutt[3], Peisen Huang[4,5], Jun Xu[1,2], Li Wang[1,2], Yang Zhou[6], Jiandong Liu[1,2], Li Qian[1,2*]

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The advent of cellular reprograming has pushed the boundaries of cell and molecular biology. Specifically, studies of cardiac reprogramming – the conversion of fibroblasts to cardiomyocyte-like cells – have delivered provocative findings on common notions of cell identity and promise for potential clinical application. At the core of most cardiac reprogramming approaches lie three transcription factors: MEF2C, GATA4, and TBX5 (MGT). Exogenous expression of MGT can convert fibroblasts into cardiomyocyte-like cells, but it is unknown if activation of endogenous MGT can mediate reprogramming or if these reprogrammed cells would have different features. In this study, we implemented CRISPR-based gene activation (CRISPRa) for targeted activation of endogenous MGT in human fibroblasts. We screened a total of 57 guide RNAs per gene with maximal transcriptional activation ranging from 100-10,000X greater than the non-targeted control. In combination with miR-133 overexpression, CRISPRa of endogenous MGT induced Troponin T and αActinin expression fourteen days after infection. Multiple combinations of guide RNAs were capable of mediating reprogramming with varying efficiencies. Finally, we simplified the CRISPRa platform by combining the MGT guide RNAs into a single, multiplex viral vector. Cellular and molecular studies of this new reprogramming model will provide mechanistic insights into cell fate conversion. Additionally, our CRISPR-based method for human cardiac reprogramming has the potential to move cardiac reprogramming closer to clinical application.

Functional Evaluation of CYLD in HPV+ Head and Neck Squamous Cell Carcinoma.

Andrew C. Prince[1], Damir Alzhanov[1], Natalia Issaeva[1,2,3], Wendel G. Yarbrough[1,2,3]

[1]Department of Otolaryngology/Head and Neck Surgery, UNC, Chapel Hill, NC, [2]Lineberger Cancer Center, UNC, Chapel Hill, NC, [3]Department of Pathology and Lab Medicine, UNC, Chapel Hill, NC

The human papilloma virus (HPV) is exacerbating the incidence of head and neck squamous cell carcinoma (HNSCC). Reminiscent of HPV-positive (HPV+) and HPV-negative (HPV-) HNSCCs' differing etiologies are their unique genotypic mutation patterns, patient demographics, and clinical outcomes. Specifically, most HPV+ HNSCCs are significantly more sensitive to chemoradiation therapy (CRT) than HPV- HNSCC. However, though disease free, patients are burdened with severe treatment-related toxicities. Thus, there is demand for CRT de-escalation while maintaining clinical outcomes. Best practice requires a "prognostic" biomarker to stratify patients. Utilizing the Cancer Genome Atlas (TCGA), genomic differences between HPV+ and HPV- HNSCC tumors were appraised. Two members of the NF-kB and innate immunity pathways, CYLD and TRAF3, exhibited significantly higher mutation rates in HPV+ compared to HPV- HNSCC. Kaplan-Meier evaluation of

HPV+ HNSCC by CLYD and TRAF3 mutation status demonstrates significantly improved 5-year survival of mutant compared to wild-type tumors. Closer evaluation of CYLD demonstrates frequent missense and truncating mutations, which are predicted to result in function loss. Given CYLD's negative NF-kB regulation, loss would increase NF-kB activity, a known oncogenic driver in Epstein-Barr virus and HIV associated tumors. We hypothesize that TRAF3 and CYLD mutational status represents a prognostic biomarker to identify patients suitable for CRT de-escalation. However, further evaluation is necessary before proceeding with clinical translation. Our lab developed a CYLD CRISPR-Cas9 knockout cell line that exhibits constitutive NF-kB activity and CYLD mutated expression vectors that reflect TCGA identified mutations. With subsequent cellular delivery, we will assess changes in NF-kB activity and perform other functional validations of created CYLD mutants.

Genotype – kinome Guided Development of Precision EGFR-Targeted Therapeutics for Glioblastoma

Erin Smithberger[1,4], Abigail Shelton[1,4], Madison Butler[1], Allie Stamper[6], Ryan E. Bash[1,4], Steven P. Angus[5], Mike East[2], Gary L. Johnson[2,3], Michael E. Berens[6], Frank B. Furnari[7], and C. Ryan Miller[1,4]

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Glioblastoma (GBM), an aggressive primary brain tumor, has poor survival and limited treatment options. However, it is an attractive candidate for precision therapeutics due to frequent amplification and/or activating mutations in the epidermal growth factor receptor (EGFR) gene and the availability of brain-penetrant EGFR tyrosine kinase inhibitors (TKI). We used molecular profiling of a genetically engineered mouse astrocyte panel to examine whether EGFR and PTEN status could be used to identify kinases upregulated in specific mutational backgrounds. Using RNA-seq and multiplexed inhibitor bead/mass spectrometry (MIB-MS) to analyze the kinase transcriptomes and proteomes, we identified potential targets for combination therapy. Wild-type EGFR overexpression in immortalized, *Cdkn2a*$^{-/-}$ astrocytes resulted in mild kinome rewiring. Excluding EGFR, only 13 kinases were overexpressed on the transcript or protein levels including *Hck*, a kinase involved in cell survival, proliferation, adhesion, and migration. Conversely, overexpression of EGFRvIII, a constitutively active EGFR extracellular domain truncation mutant resulted in significant kinome alteration – 94 kinases showed differential expression, with 43 upregulated. One attractive target was *Cdk6*, a drug-targetable, prognostically significant cyclin-dependent kinase implicated in proliferation, migration, and invasion. Finally, overexpression of EGFRvIII in cells lacking *Pten* dysregulated 66 kinases, including 35 upregulated. One interesting target in these cells was *Ddr2*, a tyrosine kinase involved in migration, invasion, and extracellular matrix remodeling. We conclude that Hck, Cdk6, and Ddr2 represent attractive therapeutic targets in their relevant genetic contexts. These findings suggest that molecular diagnostics for EGFR and PTEN status may be useful in guiding development of rational, EGFR TKI-centric drug combinations.

Plasminogen Deficiency Mitigates Liver Damage Associated with Diet-Induced Obesity, but Does Not Alter Weight Gain

Woosuk Steve Hur[1,2,3], Y-Van Nguyen[1,2,3] and Matthew J. Flick[1,2,3]

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Obesity is global health problem with 40% of the world population being classified as overweight (BMI > 25) and 13% as obese (BMI > 30). Obesity drives chronic metabolic inflammation leading to metabolic syndrome, cardiovascular disease, fatty liver disease, Type II diabetes, and certain cancers. Recently, we have shown that the blood clotting matrix protein fibrin accumulates within obese adipose tissue and liver of patients and mice challenged with a high fat diet and colocalizes with inflammatory macrophages. Previous studies showed that mice expressing a fibrinogen variant that does not engage macrophages through the integrin receptor □ M□ 2 revealed these animals had reduced weight gain and associated pathologies (*e.g.,* fatty liver disease, insulin resistance) following high fat diet (HFD)-challenge. Here, we tested the hypothesis that elimination of the fibrinolytic protease plasmin(ogen) would increase HFD-driven fibrin deposition and exacerbate macrophage accumulation, weight gain, and obesity-associated pathologies. Contrary to our hypothesis, plasminogen-deficient ($Plg^{-/-}$) mice gained as much weight as the control mice after 20 weeks on HFD. However, whereas the liver mass of HFD-challenged control mice was significantly higher than that of mice fed a control diet (CD), the livers of HFD-fed $Plg^{-/-}$ mice had a mass comparable to CD-fed mice. Plasma ALT and liver triglyceride levels were significantly lower in HFD-fed $Plg^{-/-}$ mice compared to HFD-fed control mice. Additionally, glucose clearance was more efficient in HFD-fed $Plg^{-/-}$ mice compared to control mice in a glucose tolerance test. Collectively, our data suggest that plasmin(ogen) contributes to HFD-induced fatty liver disease and glucose dysmetabolism.

List of Poster Presentations by Predoctoral Students

Posters can be previewed using the link on the Schedule page. The Zoom links below will be live only during the poster session.

POSTER SESSION I

1. *Predicting Disease States in ANCA Vasculitis Based on Dynamic Changes in Frequency of Suppressive T Regulatory Cells*
   Christian Agosto-Burgos, Dominic Ciavatta, Charles J. Jennette, Ronald Falk and Meghan Free
   https://zoom.us/j/98339978072?pwd=RE1oY3dYYTNFNmVETXF0WStqTVlOZz09
   Meeting ID: 983 3997 8072 Passcode: 938664

2. *SGK1 And SGK3 Control the Antiviral Response through Phosphorylation of NEMO at S178*
   Johnny Castillo, Ricardo Antonia, Robert Hagan and Albert S. Baldwin
   https://zoom.us/j/96326888922?pwd=UzdDRlkzZUVqRUk1SndrYWptYlVldz09
   Meeting ID: 963 2688 8922 Passcode: 957054

3. *Identifying Genes that Regulate Fibrinogen Expression*
   Dre'Von A. Dobson, James R. Byrnes, Lori A. Holle, Alisa S. Wolberg
   https://zoom.us/j/7574664637?pwd=YlYyOTAxSU56TnhrR2piSTR2NlZRQT09
   Meeting ID: 757 466 4637 Passcode: 586624

4. *Intra-tumoral Heterogeneity and Plasticity in Basal-like Breast Tumors*
   Cherise R. Glodowski, Aatish Thennavan, Susana Garcia Recio, Kevin R. Mott, Joseph Garay, Daniel Hollern, Denis Okumu, Gary L. Johnson, Charles M. Perou
   https://zoom.us/j/8196811704?pwd=Y01IVjBqTC9EVjVxV3p3L3NrWVlLQT09
   Meeting ID: 819 681 1704 Passcode: 523453

5. *Distinct Roles for Different p53 Gain-Of-Function Variants in Oral Carcinogenesis*
   Bethany L. Wagner, Kevin M. Byrd, Natalie C. Piehl, Megan T. Hastings, Melissa X. Du, and Scott E. Williams
   https://zoom.us/j/92948620009?pwd=ZWQzMFFVazlrMS8rckNmT0JVanJqQT09
   Meeting ID: 929 4862 0009 Passcode: GoHeels

6. *Mutational Signature Analysis by Targeted Sequencing in African American and non-African American Women with Breast Cancer*
   Markia A. Smith, Melissa A. Troester, Katherine A. Hoadley
   https://zoom.us/j/5619783414?pwd=REFEbXZqTWdxQklCLzNPSHR2SXV3dz09
   Meeting ID: 561 978 3414 Passcode: 950786

7. *Investigating the Pathologic Role of Normal and Low-Density Neutrophils in ANCA Glomerulonephritis*
   Dominique N. Munson, Dominic J. Ciavatta, Ronald J. Falk
   https://zoom.us/j/91384630185?pwd=ei95eE9CZjg1TDRPYlRLMDl4dW1XQT09

POSTER SESSION II

1. *The Roles of GRAF1 and GRAF2 in Maintaining Muscular Metabolism and Structure*
   Matthew Combs, Qiang Zhu, Xue Bai, Joan Taylor
   https://zoom.us/j/94291786660?pwd=ek10a1ZLUUdEaE1Rcy9GNlJOaDZHZz09
   Meeting ID: 942 9178 6660 Passcode: 129934

2. *Monocyte Heterogeneity: Its role as a Biomarker and Driver of ANCA Vasculitis*
   Carolina A. Herrera, Dominic Ciavatta, Meghan Free, Charles J. Jennette, Ronald J. Falk
   https://zoom.us/j/93841177162?pwd=OVdsdTlxd3dmMnhiY0Zza0tmNlhFQT09
   Meeting ID: 938 4117 7162 Passcode: 231540

3. *Therapeutic Potential of Factor XIII Reduction in the Context of Genetic Hypercoagulability (Factor V Leiden)*
   Emma G. Bouck, Lori A. Holle, Alisa S. Wolberg
   https://zoom.us/j/4046869484?pwd=TVFQMjhqRFJ4ZGtnOThKQ29sQklpUT09

4. *Drug Resistance in Medulloblastoma Addressed with OLIG2 inhibitor, CT-179*
   Taylor Dismuke, Chaemin Lim, and Timothy Gershon
   https://zoom.us/j/92441790755?pwd=UlJKNU9YRjAzK0lxYkFsc3hwaC9BQT09
   Meeting ID: 924 4179 0755 Passcode: 893650

5. *Association of the Immune Microenvironment and Race in the Carolina Breast Cancer Study*
   Alina M. Hamilton, Linnea Olsson, Andrea Walens, Benjamin C. Calhoun, Katherine A. Hoadley and Melissa A. Troester
   https://zoom.us/j/99530102072?pwd=WjJJYXgrT0h3SkRhWjI1RWVJNmZ1QT09
   Meeting ID: 995 3010 2072 Passcode: 418121

6. *G-protein Coupled Receptor (GPCR) and Immunoreceptor Tyrosine-based Activation Motif receptor (ITAM) Signaling in Platelets are Critical for Venous Thrombogenesis in Mice*
   Jean Marie Mwiza, Alisa S. Wolberg and Wolfgang Bergmeier
   https://zoom.us/j/92242180286?pwd=bHdSb1Zya2hrRGZGTGYxV1Nhd3EvUT09
   Meeting ID: 922 4218 0286 Passcode: DPLM

7. *Investigating the Emerging Role of B cells in Tumor Microenvironment in Triple Negative Breast Cancer*
   Xiaolu Pan, Alex Robeson, Nuo Xu, Sarah Vick, Sonia Laurie, Johnathan Serody
   https://zoom.us/j/7543158092?pwd=NUFyOWFBS0VOSUx6RjQ3Si9LQkRrQT09
   Meeting ID: 754 315 8092 Passcode: 123456

POSTER SESSION I

Predicting Disease States in ANCA Vasculitis Based on Dynamic Changes in Frequency of Suppressive T Regulatory Cells

Christian Agosto-Burgos[1,2], Dominic Ciavatta[1,2,3], Charles J. Jennette[1,2], Ronald Falk[1,2,4] and Meghan Free[1,2,4]

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Anti-neutrophil cytoplasmic autoantibody (ANCA) vasculitis is an autoimmune disease with remitting and relapsing stages and currently, no reliable biomarker can accurately differentiate between active and remission disease states. In patients with active disease T regulatory cells (Tregs) are dysfunctional, characterized by impaired suppressive capacity. However, previous studies examined Tregs as a singular population and did not investigate the heterogeneous subpopulations. Therefore, we hypothesized that dynamic changes in the frequency of highly suppressive Treg subsets can predict relapse or remission in ANCA vasculitis. To this end, we used mass cytometry to profile, in an unbiased fashion, the heterogeneity of Treg populations in healthy controls and patients with active disease and in remission. We developed a custom mass cytometry panel consisting of 28 surface and intracellular markers that have been previously reported to identify highly suppressive Treg subsets. CD4+ T cells were isolated from cryopreserved PBMCs using the EasySep™ Human CD4+ T cell Enrichment kit (STEMCELL™), stained with 28 surface and intracellular antibodies conjugated with metals, and data was acquired and analyzed using a Helios CyTOF and Cytobank, respectively. Our preliminary data suggest that the frequency of CD39+ and CCR7+ Tregs are reduced in patients when compared to healthy controls. In addition, our preliminary studies suggest that HLADR+ and CXCR3+ Tregs undergo phenotypical changes that increases their diversity in ANCA vasculitis. This project determines whether dynamic changes in the frequency of Treg subsets could be utilized to determine disease activity in ANCA vasculitis and as a foundation for future therapies.

SGK1 And SGK3 Control the Antiviral Response Through Phosphorylation of NEMO at S178

Johnny Castillo[1,2], Ricardo Antonia[3], Robert Hagan[4] and Albert S. Baldwin[1,2]

[1]Department of Pathology and Laboratory Medicine, The University of North Carolina, Chapel Hill, NC, [2]UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC, [3]University of California at San Francisco, CA, [4]Marsico Lung Institute, The University of North Carolina, Chapel Hill, NC.

Activation of the innate immune response is the first line of defense against all viruses, but the way that activation happens and how it is sustained and resolved is not fully understood. It is known, however, that upon infection viruses release their genetic material into the cellular cytoplasm activating antiviral responses through the RIG-I pathway (RNA viruses) or the STING pathway (DNA viruses). Both pathways promote

TBK1 activation and subsequent interferon beta (IFNβ) production through IRF3/7 phosphorylation. In the end the production of interferon will lead to the activation of JAK-STAT pathway which will increase transcription of genes related to viral translation inhibition, immune cell activation, apoptosis, among many other processes aiming to eliminate the viral infection. The NF-κB Essential Modulator (NEMO) has been reported to interact with TBK1 and to be required for its activation in response to double stranded RNA and double stranded DNA viral infection. Yet, the molecular mechanism by which NEMO is directed to interact with TBK1 upon viral infection has not been described. Some reports have identified Serum and Glucocorticoid Kinase 1 (SGK1), an effector of the PI3K pathway, as a negative regulator of the RIG-I pathway, an interactor of NEMO and as a target gene of phospho-STAT3. Here we found that inhibition of SGK1, and closely related kinase SGK3, increases phosphorylation of IRF3, subsequent production of IFNβ mRNA and TBK1 total levels in response to synthetic double stranded RNA (Poly I:C) in Raw264.7 cell. Moreover, we found that SGK1/3 phosphorylate NEMO at S178 *in vitro*. It is currently unclear if phosphorylation of NEMO at S178 by SGK1 is part of a feedback mechanism to prevent an extreme production of IFNβ in response to dsRNA. To address this question, we hypothesize that SGK1/3 limit TBK1 activity and subsequent interferon production and antiviral response through phosphorylation of NEMO at S178.

Identifying Genes that Regulate Fibrinogen Expression

Dre'Von A. Dobson, James R. Byrnes, Lori A. Holle, Alisa S. Wolberg
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Introduction: Fibrinogen has been shown to be causative in the pathogenesis of many diseases, including Alzheimer's disease, obesity, cancer, infections, venous thromboembolism (VTE), and ischemic stroke. Elucidating mechanisms that regulate fibrinogen expression will improve the understanding of how fibrinogen contributes to these pathologies. Recently, 41 loci associated with differential fibrinogen expression were identified in a GWAS meta-analysis cohort study (de Vries et al, 2016, Hum. Mol. Genet.). Our objectives were to establish an in vitro model of fibrinogen expression and use this to identify genes within the 41 loci that modulate fibrinogen synthesis. **Methods**: HepG2 hepatocellular carcinoma cells were transfected with siRNAs corresponding to candidate genes in each locus and cultured in the absence or presence of interleukin-6 (an acute phase stimulant). Effects on fibrinogen expression were assessed using western blotting of culture supernatants and RT-qPCR. **Results**: The siRNA-mediated knockdown of control genes (GAPDH) did not alter fibrinogen gene or protein expression. However, siRNAs targeting the fibrinogen chains decreased chain-specific mRNA expression and consequently, fibrinogen protein expression into the supernatant. Knockdown of several of the tested loci resulted in significant changes in fibrinogen protein expression. **Conclusions**: We have established an in vitro model that can detect changes in fibrinogen expression in response to known and novel genetic regulators. Using this model, we will investigate the effects of candidate genes within the identified loci on fibrinogen expression. Our studies will define mechanisms mediating fibrinogen expression and may reveal strategies to therapeutically alter fibrinogen expression in disease.

Intra-tumoral Heterogeneity and Plasticity in Basal-like Breast Tumors

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Triple Negative Breast Cancer (TNBC) is an aggressive malignancy with a poor prognosis that accounts for 10-20% of breast cancer cases worldwide. Tumor resistance to chemotherapy is a major obstacle facing patients with TNBCs, as TNBCs lack conventional druggable targets and patients rely on chemotherapy as the main treatment option. Intra-tumoral heterogeneity and tumor cell plasticity are thought to contribute to resistance to chemotherapy. More than 70% of TNBCs subtype as basal-like breast cancers (BLBCs), which are characterized by high intra-tumoral heterogeneity and diverse tumor cells. Currently, the mechanism by which BLBC tumor cells develop resistance to chemotherapy is poorly understood. This work aims to identify the genetic regulators of plasticity between subpopulations of BLBCs and to test whether targeted agents blocking or initiating this plasticity can lead to changes in tumor chemo-sensitivity. To characterize the heterogeneity of epithelial cells in BLBC tumors, we performed flow cytometry and single cell RNA-sequencing (scRNA-seq) on multiple, independently arisen p53-null murine BLBC tumors. We found clear intra-tumoral heterogeneity with distinct cellular subpopulations present within most BLBC tumors tested thus far. We are currently using gene expression and network analyses to identify possible genetic regulators of each subpopulation. Further, we are testing chromatin-remodeling inhibitors and chemotherapies on murine BLBCs to measure transcriptional response to therapy. The goal is to determine if these cellular subpopulations change frequency or phenotype with treatment. Ultimately, identifying regulators and targeted agents that modulate cellular plasticity and chemo-sensitivity would lead to improved therapeutic regimens for patients with chemo-resistant TNBCs.

Distinct Roles for Different p53 Gain-of-function Variants in Oral Carcinogenesis

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The tumor suppressor p53 (*TP53*) is mutated in about two-thirds of head and neck squamous cell carcinomas (HNSCCs) and correlates with poorer prognosis. While most tumor suppressors are inactivated in cancer, mutations in *TP53* occur in hotspots and can lead to gain-of-function. We are investigating how p53 alterations impact oral epithelia (OE) homeostasis, differentiation, and response to genotoxic stress in order to better understand the role of specific p53 variants in HNSCC. Using mouse models of two p53 hotspot mutations — R270H (human R273H) and R172H (human R175H) — and RNA-seq, we assessed the transcriptional effects of these mutations in epidermis and OE. The

expression of genes involved with differentiation and cell fate decisions were altered in R172H mutants, while genes involved in DNA damage repair, cell cycle, and genome maintenance were upregulated in R270H mutants. In R172H mutants, these transcriptional changes manifest as altered division orientation and aberrant cell fate choices as revealed by genetic lineage tracing in normal and dysplastic OE. On the other hand, R270H mutants show no obvious changes in fate decisions, but display a compromised DNA damage response. As in humans, in oral tumorigenesis assays in mice, both p53 mutants develop tumors about 50% sooner and show reduced survival compared to their wildtype littermates, demonstrating that although each p53 mutant has different effects on cellular processes, they are similarly detrimental in the context of tumor initiation. Together, these studies illuminate the distinct roles that different p53 mutants play in OE stem cell fate decisions and their impact on tumorigenesis.

Mutational Signature Analysis by Targeted Sequencing in African American and Non-African American Women with Breast Cancer

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Background: Mutational signatures in breast cancer have been well-studied in populations of mostly Caucasian women. Our lab has shown that Black women are more likely to develop aggressive subtypes including basal-like and HER2-enriched tumors. While the expression data supports biological differences in tumors of Black and white women that lead to overall mortality differences, less is known about the role of mutational signatures in racial disparities. **Methods:** We combined a 1,123-gene DNA panel measured in the Carolina Breast Cancer Study (CBCS, 50% Black) and the same genes subset from The Cancer Genome Atlas (TCGA, 20% Black) WES. We used the R package SomaticSignatures to identify established and novel mutational signatures in the combined dataset and look for associations by race. **Results:** Basal-like tumors have a higher frequency of C>A and C>G mutations compared to luminal tumors with a high frequency of C>T mutations. We detected six main mutational signatures associated with APOBEC activity (79%), aging (94%), homologous recombination (74%), defective mismatch repair (85%), defective base excision repair (73%), and smoking (85%). Accuracy ranged between 73-94% compared to paired WES in TCGA. Homologous recombination signature was more prevalent in Black women, while white women had higher occurrence of aging and defective DNA mismatch repair signatures. **Conclusions:** This preliminary examination in a targeted gene panel identified mutational signatures with high accuracy. Suggestive differences in frequency of signatures by race were noted. Ongoing sequencing will increase our sample size to further examine the factors that interact with mutational signatures leading to racial disparities.

Investigating the Pathologic Role of Normal and Low-Density Neutrophils in ANCA Glomerulonephritis

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Anti-Neutrophil Cytoplasmic Autoantibody glomerulonephritis (ANCA GN) is one of the most common forms of GN in adults 50 years of age and older, however ANCA GN can occur at any age. ANCA is characterized by the production of autoantibodies against autoantigens, myeloperoxidase (MPO) or proteinase-3 (PR3). Neutrophils are the primary source of MPO or PR3 autoantigen overexpression. Activated neutrophils attack small vessels causing pathological lesions induced by ANCAs; thus, it is urgent to further investigate neutrophil's function to potentially prevent kidney injury as a treatment option. Recently, our lab reported that a unique subset of neutrophils, known as low-density neutrophils (LDNs), are elevated in patients with ANCA GN compared to healthy controls (HC) and had a high baseline activation level prior to *in vitro* stimulation with ANCAs. This suggest that the spontaneous activation of LDNs can exacerbate the autoimmune response and contribute to the ANCA GN pathogenesis. We hypothesize that the immunophenotype and transcriptional profile of LDNs differs from NDNs and correlates with disease activity in ANCA GN. To test this hypothesis, we will use flow cytometry and expression analysis with qPCR comparing paired LDNs and NDNs from patients to identify differential activation states. Based on our preliminary data, LDNs express surface markers ($CXCR4^{high}$, $CD62L^{low}$) and genes ($FcyR\text{-}IIA^{high}$, MPO^{high}, $PRTN3^{high}$) in ANCA GN patients, which suggest that LDNs are activated and signaled to migrate into injured tissue. Also, patients have a higher frequency of activated LDNs ($CD62L^{low}$) compared to HC; therefore, activated LDNs may contribute to the ANCA GN pathogenesis.

POSTER SESSION II

The Roles of GRAF1 and GRAF2 in Maintaining Muscular Metabolism and Structure

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The maintenance of muscle quality is critical for both mobility and whole-body metabolism. This fact is underscored by findings that defects in muscle function due to genetic disorders, nutrition, aging, and/or a sedentary lifestyle can have a major impact on a variety of disorders ranging from cardiovascular to cancer to mental health disease. Thus, discovering new ways to attenuate muscular defects would provide a great benefit to public health. Our lab has generated data which suggests that GRAF1 and GRAF2 are regulators of Parkin-dependent mitophagy. Accordingly, the objective of this study is to implicate GRAF1 and GRAF2 as novel therapeutic targets for muscular defects by characterizing the functional effects of GRAF depletion in mice. To accomplish this, we performed Western blotting with wild-type and GRAF-deficient mouse striated muscle lysates to measure mitophagy-associated protein levels. We also performed succinate dehydrogenase and laminin staining in mouse skeletal muscle tissues to determine oxidative capacities and cross-sectional areas, respectively. We found that GRAF depletion causes Parkin accumulation and decreased LC3 turnover. Furthermore, our preliminary findings are that GRAF2 depletion leads to decreased oxidative capacity and increased cross-sectional areas. Therefore, we conclude that GRAF depletion causes mitophagy defects which lead to accumulation of damaged mitochondria and muscle fiber hypertrophy. This indicates that GRAF1 and GRAF2 are important regulators of both muscle energetics and muscle structure. We are currently engaged in elucidating the mechanisms which underlie the roles of GRAF1 and GRAF2 that we have characterized.

Monocyte Heterogeneity: Its Role as a Biomarker and Driver of ANCA Vasculitis

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Anti-neutrophil cytoplasmic autoantibody (ANCA) vasculitis is a chronic relapsing and remitting autoimmune disease characterized by systemic vasculitis and tissue necrosis, with 20-25% of patients progressing to end-stage renal disease. Autoimmune diseases involve dysregulation of the immune system and complex interactions among various immune cell types. Monocytes are primary sources of ANCA autoantigens and when activated by ANCA, cause systemic blood vessel damage. Three monocyte subsets exist in peripheral blood (classical $CD14^{+}CD16^{-}$, intermediate $CD14^{+}CD16^{+}$, and non-classical $CD14^{dim}CD16^{+}$) and the frequencies of these subsets are altered in inflammatory diseases. However, how monocyte subsets contribute to ANCA vasculitis is unknown. We hypothesize that different monocyte subsets impact disease and indicate relapse and remission. Monocytes were isolated from peripheral blood of healthy controls (n=7), ANCA vasculitis patients (n=19), and lupus nephritis patients (n=6). Monocytes were also isolated from paired peripheral blood

and urine of patients with ANCA vasculitis (n=11) and lupus nephritis (n=6). Using flow cytometry, we found that intermediate monocytes, which have a proinflammatory phenotype, are elevated in the peripheral blood in a subset of ANCA vasculitis patients. Furthermore, in patients with ANCA vasculitis and lupus nephritis, the percentage of total $CD14^+$ monocytes was significantly elevated ($p=0.0002$) in urine compared to peripheral blood. Elevated $CD14^+$ monocytes in the urine suggests their migration from the circulation to sites of inflammation and may reflect disease activity. Defining how monocyte subsets contribute to disease will identify biomarkers of disease activity and can inform treatment of ANCA vasculitis.

Therapeutic Potential of Factor XIII Reduction in the Context of Genetic Hypercoagulability (Factor V Leiden)

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Background: Venous thromboembolism results from aberrant activation of the coagulation cascade, which culminates in fibrin polymerization. Fibrin crosslinking by Factor XIIIa promotes red blood cell retention in thrombi; FXIII-deficient mice form smaller thrombi *in vivo* because of impaired RBC retention. The therapeutic utility of reducing FXIII activity in settings of hypercoagulability is unknown. ***Objectives:*** (1) Establish human and mouse *in vitro* models of Factor V Leiden (FVL)-associated hypercoagulability. (2) Test the potential of FXIII inhibition to reduce FVL hypercoagulability. ***Methods:*** Clotting was triggered in human normal pooled plasma or heterozygous FVL pooled plasma; thrombin generation was measured by cleavage of a fluorogenic substrate, and fibrin formation was monitored by turbidity. Fibrin formation was performed in plasma from $F5^{+/+}$, $F5^{+/L}$, and $F5^{L/L}$ mice. Assays were performed in the presence of thrombomodulin to reveal the hypercoagulable phenotype. ***Results:*** Addition of thrombomodulin to human normal pooled plasma resulted in a dose-dependent decrease in thrombin generation and fibrin formation. Plasma from human heterozygous FVL carriers displayed resistance to the thrombomodulin-dependent decrease in thrombin generation and fibrin formation. Similarly, addition of thrombomodulin to plasma from $F5^{+/+}$ mice resulted in decreased fibrin formation and plasmas from $F5^{+/L}$ and $F5^{L/L}$ mice were resistant to this effect. ***Conclusions and future directions:*** Plasma from human and mouse carriers of FVL demonstrated the expected resistance to thrombomodulin in thrombin generation and fibrin formation assays. The impact of FVL on whole blood clot weight needs to be characterized before testing the utility of FXIII inhibition in ameliorating the FVL hypercoagulable phenotype.

Drug Resistance in Medulloblastoma Addressed with OLIG2 Inhibitor, CT-179

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Medulloblastoma is the most common malignant pediatric brain tumor. Medulloblastoma patients need treatment options, because conventional therapy (i.e. surgical resection, chemotherapy, and radiation) leaves survivors at risk of neurocognitive injury, growth defects, and psychosocial impairment. We seek to identify novel treatments that will address systemic toxicity and tumor recurrence. Our work on targeted therapies, principally using the CDK4/6 inhibitor palbociclib (POx-palbo) has shown that medulloblastomas consistently develop resistance and that this resistance is driven by OLIG2+ stem cells. Importantly, OLIG2-expressing stem cells have also been shown to drive recurrence after conventional therapy. Based on these data, we propose that tumors respond to the initial suppressive effect of diverse therapies by increasing the pool of Olig2+ stem cells, and that resistance can be address by directly targeting this population. We therefore examined the potential efficacy of CT-179, a first-in-class OLIG2 inhibitor, in transgenic mice that develop medulloblastoma. In our luciferase reporter medulloblastoma model, CT-179 treatment decreased sonic hedge-hog pathway signaling resulting in decreased tumor growth. Dynamic flow cytometric studies show that CT-179 alters cell-cycle occupation and promotes a shift towards cell-cycle arrest. Additionally, a Kaplan-Meir curve of CT-179 administration showed a significant extension of survival in tumor mice. We propose the combinational therapy of CT-179 and POx-palbo will lead to newly effective medulloblastoma treatment addressing both toxicity and heterogeneous response to therapy.

Association of the Immune Microenvironment and Race in the Carolina Breast Cancer Study

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Black women suffer 40% higher mortality from breast cancer (BC) compared to non-Hispanic white women. Evidence supports the importance of the immune microenvironment in BC survival, but immune response differences by race are poorly understood. Therefore, we sought to evaluate how phenotypes of immune response vary across race and tumor subtype. We curated a 48-gene panel representative of 13 individual immune cell types and performed gene expression profiling on tissue from 1957 BC patients, including 1033 Black and 924 Non-Black women from the Carolina Breast Cancer Study. Consensus clustering was used to identify phenotypes of immune response, and we estimated associations with immune infiltrates, BC intrinsic subtype, risk-of-recurrence (ROR-PT) scores and race, adjusting for age and tumor stage. We identified three BC immune phenotypes defined by features related to an Adaptive-enriched, Innate-enriched, or Immune-quiet microenvironment. These expression-based classes correlated with protein-based quantification of immune cells from

corresponding slides. Both Adaptive-enriched and Innate-enriched tumors were associated with high ROR-PT scores, the basal-like intrinsic subtype and Black race. Within the Adaptive-enriched class, Black women displayed decreased CD8 T cell scores and increased T-reg scores relative to Non-Black women. Taken together, immune response appears to be intricately related to race and tumor subtype, with black women having strong associations with adaptive-enriched and innate-enriched immune microenvironments. Differences in CD8 T cell and T-reg expression suggest that even within broad classes of immune response, racial differences in specific cell-type distributions exist. These immune response differences may be targetable to help close the racial disparity gap in BC.

G-protein Coupled Receptor (GPCR) and Immunoreceptor Tyrosine-based Activation Motif receptor (ITAM) Signaling in Platelets are Critical for Venous Thrombogenesis in Mice

Jean Marie Mwiza, Alisa S. Wolberg* and Wolfgang Bergmeier*
*University of North Carolina at Chapel Hill, Chapel Hill, NC *Contributed equally to this work*

Venous thrombosis (VT) affects close to a million Americans every year. Thrombi contain platelets but little is known about if and how they affect venous thrombogenesis. We hypothesized that deficiency or inhibition of important platelet agonist receptors impairs venous thrombogenesis in mice. We seek to investigate the role of platelet ITAM receptors and GPCRs for venous thrombogenesis in mice. VT was induced by partially ligating the inferior vena cava (IVC stenosis), in mice deficient in either GPCR or ITAM signaling. Mice were sacrificed after 48hrs of flow restriction and thrombus weights determined. To study early stages of VT development, the saphenous vein was ligated and intravital imaging of platelets and leukocytes was performed 2hrs post flow restriction. Thrombus consolidation was evaluated *ex vivo* in whole blood clot contraction studies. Our studies demonstrate that deficiency in ITAM signaling significantly reduced VT development in mice. Platelet-leukocyte adhesion to the venous wall was significantly reduced during the early phase of VT. In vivo clots after 48 hrs of flow restriction of the IVC were significantly reduced and in vitro clots from mice deficient in both GPCRs and ITAM receptors were smaller than in the control. We therefore report here that venous thrombogenesis in mice depends on both ITAM and GPCR signaling in platelets. Mechanistic studies on the role of these platelet signaling are ongoing.

Investigating the Emerging Role of B cells in Tumor Microenvironment in Triple Negative Breast Cancer

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Checkpoint inhibitor therapy (CPIT) empowers the immune cells by blocking the immune checkpoints inhibitor like PD-1 in T lymphocytes. However, the response rate of CPIT is still low in many malignancies like triple negative breast cancer (TNBC). Further investigating mechanisms of immune cells helps to improve the immune therapy. Multiple groups have reported important PD-1 function in B cells. Our group has shown that peritumoral B cells indicate the outcome of patients with TNBC. The clinical response to CPIT correlated most strongly with numbers of B cells in the

tumor microenvironment. Here, we hypothesized that PD-1 expression restrains B cell production of cytokines and proliferation and induces metabolic changes in B cells in the TME. Blocking the function of PD-1 with monoclonal antibody will, therefore, reverse these effects. To validate our hypothesis, we designed an *in vitro* B cell culture system to evaluate the PD-1 function in B cells. We harvested the splenic cells in wild type mice. We isolated B cell by sorting for CD19+ positive population. B cell was cultured *in vitro* and activated by activators like anti-IgM, CpG, or lipopolysaccharide (LPS). We assessed the B cell livability and activity by flow cytometry. Isolated B cells treated with Anti-IgM + CD40L + IL4 are shown the more livability compared with other treatment conditions. PD-1 expression level is upregulated in B cells with anti-IgM treatment compared with control with no stimulation. We will block the PD-1 expression in later experiments to validate our hypothesis.

List of Poster Presentations by Residents, Postdoctoral Fellows, and Clinical Fellows

Posters can be previewed using the link on the Schedule page. The Zoom links below will be live only during the poster session.

POSTER SESSION I

15. *New Aspects in HVP-Dependent Oropharyngeal Squamous Cell Carcinomas Development*
Damir Alzhanov, Natalia Issaeva, Wendel G. Yarbrough
https://zoom.us/j/95965320891?pwd=SEdMN2NCNnNMNlY3QUY5emFVODkyQT09
Meeting ID: 959 6532 0891 Passcode: 050395

16. *Next Generation Sequencing Reveals Novel Mutations in a Collision Tumor of Glioblastoma and Meningioma*
Kelly Chamberlin, Gregory Chamberlin, Katherine Saunders, and Simon Khagi
https://us02web.zoom.us/j/6933090123?pwd=QkwrOHFPZXB0K3prVzljVGRYd3FUZz09
Meeting ID: 693 309 0123 Passcode: 2e47Qu

17. *Significant Blood use Reduction with Improvement of Outcome Sweet Fruit from 5 Years of Focused Patient Blood Management with Orthopedic Surgery*
Erin Garrett and Walter Linz
https://zoom.us/j/92267918946?pwd=SFlKaXV2T0V6WHpkdGVTdVhRejRZUT09
Meeting ID: 922 6791 8946 Passcode: 745765

POSTER SESSION II

18. *Validation of ddPCR-quantified Standards for Use in Calibrating Viral Load Measurements by NGS*
Derek Hoerres, Qunsheng Dai, Sandra Elmore, Sid Sheth, Gaorav P. Gupta, Sunil Kumar, Nathan Montgomery, Margaret L. Gulley
WITHDRAWN

19. *Molecular Mechanisms of Therapeutic Demethylation on HPV Genes in HPV+ Head and Neck Cancer*
Hina Rehmani, Asel Biktasova, Michael Hajek, Wendell G. Yarbrough, Natalia Issaeva
https://zoom.us/j/95965320891?pwd=SEdMN2NCNnNMNlY3QUY5emFVODkyQT09
Meeting ID: 959 6532 0891 Passcode: 050395

POSTER SESSION I

New Aspects in HVP-Dependent Oropharyngeal Squamous Cell Carcinoma Development

Damir Alzhanov[1], Natalia Issaeva[1,2,3], Wendel G. Yarbrough[1,2,3]
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The incidence of human papilloma virus HPV- associated head and neck cancer (HPV+ HNC) in the US has dramatically increased in the last decade, and has now surpassed that of cervical cancer, highlighting the need to better understand the etiology of HPV+ HNC. Research has focused on identification of HPV integration sites and their involvement in carcinogenesis driven by the dogma that integration drives carcinogenesis. However, recent data show that many HNCs lack integration and harbor only episomal HPV. We found that these tumors express all HPV genes leading us to hypothesize that they may make virus or be capable of transferring functional HPV genes to surrounding cells. Indeed, we identified an HPV DNA Transferring Agent (HDTA) in HPV-associated HNC. Based on our preliminary findings, we propose a novel model of HPV-driven carcinogenesis dependent on episomal HPV and production of HDTA (which may prove to be viral particles). Further molecular characterization of HDTA aim to determine if it represents a virus or specific extracellular vesicles.

Next Generation Sequencing Reveals Novel Mutations in a Collision Tumor of Glioblastoma and Meningioma

Kelly Chamberlin, MD[1], Gregory Chamberlin, MD[2], Katherine Saunders, DO[2], and Simon Khagi, MD[1,3]
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Collision tumors of multiple primary brain neoplasms are rare in patients without a neurocutaneous syndrome or history of intracranial radiation. We report such a case in a 42-year-old female with a history of seizures who presented with headaches and altered mental status. Magnetic resonance imaging revealed a single heterogeneous, rim-enhancing lesion in the left parieto-occipital, periventricular region involving the corpus callosum. Initial stereotactic biopsy demonstrated glioblastoma. The patient underwent tumor resection with histologic evidence of glioblastoma and meningioma. This case is unusual in its presentation, as the meningioma component was not identifiable on imaging or upon intra-operative examination. We are also the first to report next generation sequencing (NGS) data for both distinct tumor types within a collision tumor. The glioblastoma exhibited a deep deletion of *CDKN2A* and novel missense mutations in *TAF1L* and *CSMD3*. The meningioma component contained the same *TAF1L* mutation at a lower variant allele

frequency. NGS may yield insight into molecular drivers of intracranial collision tumors and aid in identifying future therapeutic targets.

Significant Blood use Reduction with Improvement of Outcome Sweet Fruit from 5 Years of Focused Patient Blood Management with Orthopedic Surgery

Erin Garrett and Walter Linz

[1]Transfusion Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC [2]Baylor Scott and White Memorial/Texas A&M Health Science Center, Temple, TX

Background: During the previous two decades, many studies have demonstrated that transfused blood products exert many previously underappreciated immunological risks to recipients and historic transfusion and utilization triggers were biased toward unnecessary transfusion. More recently, national patient blood management efforts have attempted to decrease excess utilization with application of best practice. Though the efforts apply to all services, this study will demonstrate the exceptional results in orthopedic surgery derived from implementing an institution wide best practice approach in transfusion medicine. **Materials/Methods:** Data was compiled from a five-year (2011-2016) single institution retrospective observational review of inpatient Orthopedic Surgical transfusion rate, utilization rate, length of stay and infectious complications data with the use of a web-based patient blood management tool (IMPACT® Online). **Results**: Since the implementation of IMPACT® Online in 2011, Transfusion rate and Utilization rate for orthopedic surgery dropped from 21.9% in 2011 to 4.3% in 2016 and 2.62 units transfused per patient to 1.6 units transfused per patient ($p<0.05$). Simultaneously, the Length of Stay dropped from 3.6 days to 3.2 days and Infection complication dropped from 3.7% to 2.62%. During this 5 year period, the transfusion guidelines changed lower the transfusion trigger from 8 g/dl of Hb to 7 g/dl Hb and default single unit transfusion emerged as the institutional policy. **Discussion**: In our study we again show that a restrictive approach to blood management had many benefits without any evidence of harm. Application of best transfusion practice prevents the exposure of patients to the immunological effects of unnecessary transfusion. In a 5-year single institution retrospective study, it was observed that both transfusion rate and utilization rate markedly decreased from baseline, while clinical benchmarks of length of stay and infection rates improved. This data supports the contention that improved transfusion practice results in improved patient outcomes.

POSTER SESSION II

Validation of ddPCR-Quantified Standards for Use in Calibrating Viral Load Measurements by NGS

Derek Hoerres[1], Qunsheng Dai[1,2], Sandra Elmore[1,2], Sid Sheth[2,3], Gaorav P. Gupta[2,4], Sunil Kumar[2,4], Nathan Montgomery[1,2], Margaret L. Gulley[1,2]

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WITHDRAWN

Molecular Mechanisms of Therapeutic Demethylation on HPV Genes in HPV+ Head and Neck Cancer

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Human papillomavirus (HPV) rates have skyrocketed to epidemic levels in the US and the HPV16 strain is responsible for approximately 80% of head and neck squamous cell carcinoma (HNSCC) in the oropharynx. Treatment involves concurrent high dose radiation and chemotherapy, which often result in painful, lifelong side effects for patients. The substantial rate of recurrence and high morbidity of primary therapy suggest that new therapies are needed. Since HPV-positive (HPV+) HNSCC display a higher methylation level compared to its HPV-negative (HPV-) counterpart, we postulated that methyltransferase inhibitors may be an effective therapeutic option. 5-azacytidine (5-aza) is an FDA approved synthetic cytidine analog that traps methyltransferases to chromatin and causes DNA demethylation. Our results show that HPV+ HNSCC cells are sensitive to 5-aza, that transcription of HPV oncogenes E6 and E7 are suppressed by 5-aza, and that 5-Aza induces DNA double-stranded breaks (DSBs) at the sites of hypomethylated DNA only in HPV+ HNSCC. Additionally, low doses of 5-aza delayed HPV+ xenograft tumor growth and that HPV+ tumors from patients decreased after 5-aza treatment in a window clinical trial. Furthermore, we identified and characterized demethylation-triggered molecular pathways that are important for cancer survival and progression and that are specific for HPV+ HNSCC. Our studies provide a basis for a new rational targeted therapy, which is desperately needed for patients with recurrent or metastatic HPV+ head and neck cancer.

Acknowledgements

The organizers of the *2020 Annual Research Symposium* would like to thank everyone who contributed to the success of this event. We appreciate the enthusiastic participation from the graduate students, residents, postdoctoral fellows, and clinical fellows who shared their research with the Department through oral and poster presentations. We thank Alina Hamilton and Angana Mukherjee for serving as moderators for the oral presentations by the graduate students and fellows. We also thank the individual faculty members who judged the presentations. We give special thanks to Dr. Broaddus for his support of this important event, and to Mrs. Angel Truesdale for her assistance with planning the event, preparation of the program booklet, and for logistical support during the event. Finally, thank you to everyone that attended this event to learn about the excellent research being conducted by our graduate students, residents, postdoctoral fellows, and clinical fellows.

J. Homeister and C. Vaziri