ALPHA ΩMEGA ALPHA

49TH ANNUAL SCIENTIFIC RESEARCH SYMPOSIUM

Friday, August 3, 2018
Great Hall
The Mary Duke Biddle Trent Semans Center for Health Education
Άξιον ωφελείν τοὺς ἀλγοῦντας

"Be worthy to serve the suffering"
ALPHA OMEGA ALPHA MEDICAL HONOR SOCIETY
49th ANNUAL SCIENTIFIC RESEARCH SYMPOSIUM

L. Ebony Boulware, MD, MPH
Professor and Chief, Division of General Internal Medicine in the Department of Medicine
Director, Clinical and Translational Science Institute
Associate Vice Chancellor for Translational Science
Duke University School of Medicine

Quest to Make a Difference: Building a Career in Translational Clinical Studies to Improve Patients’ Health

Friday, August 3rd, 2018
The Mary Duke Biddle Trent Semans Center for Health Education
Great Hall

7:30 – 8:00 AM Platform Presentation Setup
     Breakfast – Served in the Great Hall Lobby, Level 0
8:00 – 8:30 AM Keynote Address: L. Ebony Boulware, MD, MPH
8:40 – 11:00 AM Platform Presentations
11:00 – 12:45 PM Poster Presentations
12:45 – 1:30 PM Pick up lunch – Learning Hall, Level 2

RETURN TO THE GREAT HALL FOR 1:30 PM EVENTS

1:30 – 2:15PM Updates to the Curriculum
     Edward Buckley, M.D., Vice Dean for Education
     Colleen O'Connor Grochowski, Ph.D., Associate Dean for Curricular Affairs
2:15 – 2:45PM Valentine Esposito, Davison Council President
2:45 – 3:00 PM Presentation of Awards
3:00 PM Adjourn
THE DUKE AΩA CHAPTER WOULD LIKE TO THANK THE FOLLOWING FOR THEIR PARTICIPATION IN TODAY’S SYMPOSIUM

Platform Judges

Edward Buckley, M.D.
Professor of Ophthalmology; James Pitzer Gills, III, M.D. and Joy Gills Professor of Ophthalmology in the School of Medicine
Professor in Pediatrics
Vice Dean for Education
Chair, Department of Ophthalmology

Daniel Laskowitz, M.D., M.H.S.
Professor and Vice Chair of Neurology
Professor of Anesthesiology and Neurobiology
Director, Neurovascular Laboratories

Bruce Klitzman, PhD
Associate Professor of Surgery
Assistant Research Professor in Cell Biology
Associate Professor of Biomedical Engineering
Core Faculty in Innovation & Entrepreneurship

Poster Judges

Achanta, Salya, Ph.D, D.V.M., Assistant Professor in Anesthesiology
Andolsek, Kathryn, M.D., MPH, Associate Director, Graduate Medical Education; Community and Family Medicine
Ashley, Patricia, M.D., Ph.D, Assistant Professor of Pediatrics
Bennett, Ellen, Ph.D, Assistant Professor in Neurology
Bradley, Don, M.D., Consulting Professor of Community and Family Medicine
Chalian, Hamid, M.D., Medical Instructor of Radiology
Cheifetz, Ira, M.D. FCCM, FAARC, Professor of Pediatrics, Professor in Anesthesiology, Chief, Division of Pediatric Critical Care Medicine
Chi, Ashley, Ph.D, M.D., Associate Professor of Molecular Genetics and Microbiology
Chu, Vivian, M.D., Associate Professor of Medicine – Infectious Disease
Cunningham, Coleen, M.D., Professor of Pediatrics
Elmallah, Mai, MBBCH, Professor of Pediatrics
Engle, Deborah, Ed.D, M.S., Assistant Dean, Assessment and Evaluation
Fecci, Peter, M.D., Ph.D, Assistant Professor of Neurosurgery
Fraser, Matthew, Ph.D, Associate Professor of Surgery
Freedman, Neil, M.D., Professor of Medicine
Gbadebesin, Rasheed, M.D., MBBS, Associate Professor of Pediatrics and Nephrology, Faculty Member of Duke Molecular Physiology Institute
Glass, Oliver, Ph.D, Medical Instructor of Medicine
Hall, Russell, M.D., J. Lamar Callaway Professor of Dermatology
Holley, Christopher, M.D., Ph.D., Assistant Professor of Medicine – Cardiology
Kaelberer, Maya, Post Doc, Department of Medicine, Gastroenterology
Kleeberger, Steven, Ph.D, Adjunct Professor of Medicine
Klitzman, Bruce, Ph.D, Associate Professor of Surgery
Kwatra, Madan, Ph.D, Associate Professor in Anesthesiology
Limkakeng, Alexander, M.D., M.H.S., Associate Professor of Surgery
Lo, Joseph, Ph.D, Professor of Radiology, Biomedical Engineering and Electrical & Computer Engineering, Associate Vice Chair, Research Radiology
MacLeod, Amanda, M.D., Assistant Professor of Dermatology
McCall, Shannon, M.D., Associate Professor of Pathology
McNeill, Diana, M.D., Professor of Medicine, Endocrinology and Metabolism
Moon, Richard, M.D., Professor of Anesthesiology and Environmental Physiology
Naylor, Jennifer, Ph.D, Associate Professor of Psychiatry and Behavioral Sciences
Nixon, Andrew, Ph.D, Associate Professor of Medicine
Ostbye, Truls, M.D., M.P.H., Professor of Community and Family Medicine
Permar, Sallie, M.D., Ph.D., Assistant Professor of Pediatrics- Infectious Diseases, Associate Professor in Immunology, Associate Professor in Molecular Genetics and Microbiology, Member of Duke Human Vaccine Institute, Affiliate, Duke Global Health Institute
Peters, Katherine, M.D., Ph.D, Associate Professor of Neurosurgery
Rao, P. Vasantha, Ph.D., Professor Ophthalmology, Professor in Pharmacology and Cancer Biology
Rapoza, Maria, Ph.D, Assistant Professor of Medicine
Reed, Ann, M.D., William Cleland Professor of Pediatrics, Chair, Department of Pediatrics
Rynn, Moira, M.D., Consulting Professor of Psychiatry and Behavioral Science
Salama, Joseph, M.D., Associate Professor and Chief, Radiation Oncology Clinical Services, Durham VA Medical Center
Sell, Timothy, Ph.D, Associate Professor of Orthopedic Surgery
Souma, Tomo, M.D., Ph.D, Medical Instructor of Medicine
Stringham, Nicole, Ph.D, Postdoctoral Associate
Sunday, Mary, M.D., Ph.D, Professor of Pathology
Troy, Jesse, Ph.D, Assistant Professor of Pediatrics
Tucci, Debara, M.D., Professor of Surgery, Head and Neck Surgery & Communication Sciences
Velkey, Matt, Ph.D, Assistant Professor of the Practice of Medical Education in the department of Cell Biology
White, Leonard, Ph.D, Associate Professor in Neurology
Whitson, Heather, M.D., M.H.S., Associate Professor of Medicine
Wolf, Myles, M.D., Charles Johnson Professor of Medicine
Wood, Jamie, Ph.D, Assistant Professor of the Practice of Medical Education

Special Thanks
Mr. E. Arthur Palumbo, a 1949 Duke University graduate, established The Palumbo Family Medical Scholarship which provides a full-tuition scholarship for the fourth year of medical school and will be awarded today. Mr. Palumbo is a great friend of Duke Medicine who has also provided major funding to Duke Children’s Hospital, and who also established The Leonard Palumbo, Jr., MD Faculty Achievement Award in memory of his brother – a Duke University School of Medicine alumnus (MD 1944) and former Duke Obstetrics and Gynecology faculty member. The award is given annually to one or more Duke School of Medicine faculty members who best exemplify the qualities of compassionate patient care and dedication to teaching and mentoring young physicians that his late brother embodied.
8:40 – Allison Bloom  
*Biological Aging Drives Subtle Changes in the Host Genomic Response to Acute Respiratory Infection*  
Mentor: Micah McClain, MD, PhD  
Study Program: Microbiology, Immunology and Infectious Disease; Andrew Alspaugh, MD, Director  
The Eugene A. Stead Jr. MD Research Scholarship

8:51 – Kelly Buchanan  
*The Taste of Calories in the Gut*  
Mentor: Diego Bohórquez, Ph.D.  
Study Program: Molecular Medicine: Director, David Hsu, MD, PhD  
HHMI Medical Research Fellowship

9:02 – Jeffery Kwock  
*Innate Antiviral Immunity is activated upon Skin injury via an IL-27/STAT1 axis*  
Mentor: Amanda MacLeod, MD  
Study Program: Microbiology, Immunology and Infectious Disease; Director, Andrew Alspaugh, MD  
The Eugene A. Stead Jr. MD Research Scholarship

9:13 – Claire Edelman  
*Extended HPV genotyping: Comparing HPV type-distribution in self and provider-collected samples*  
Mentor: John Wilson Schmitt, MD  
Study Program: Primary Care Leadership Track, Anh Tran, PhD, MPH, Director

9:24 – Andrew Yuan  
*Examining the Validity of Computerized Movement Tracking as an Index of Symptom Severity for Autism Clinical Trials*  
Mentor: Geraldine Dawson, PhD  
Study Program: Clinical Research; Vivian Chu, MD, Director

9:35 – Matthew Lyes  
*Adipose Stem Cell Crosstalk with Chemo-residual Breast Cancer Cells: Implications for Tumor Recurrence*  
Mentor: Robin Bachelder, MD, PhD  
Study Program: Pathology; Shannon McCall, MD, Director  
The Donald B. Hackel Fellowship
9:46 – Sarah Scharber  
*Ketamine versus Midazolam for Prehospital Agitation*  
Mentor: Jon Cole, MD, Department of Emergency Medicine, Hennepin County Medical Center, Minneapolis, MN  
Study Program: Clinical Research; Vivian Chu, MD, Director

9:57 – Gireesh Reddy  
*Novel Nanoparticle Formulation of Niclosamide Treats Metastatic Human and Canine Osteosarcoma in vivo*  
Mentor: Will Eward, DVM, MD  
Study Program: Radiation, Radiation Oncology and Medical Physics; Joseph Lo, PhD, Director

10:08 – Temitope Gafaar  
*Advance Care Planning and Qualifying Death: Perceptions in Northern Tanzania*  
Mentor: Catherine Staton, MD, MScGH  
Study Program: Global Health; Director, Dennis Clements, PhD, MPH, MD  
Doris Duke International Clinical Research Fellows Program

10:19 – Anthony Lin  
*Deeply-Personalized Medicine: Bringing Deep Learning to Sepsis Care*  
Mentor: Hayden Bosworth, PhD  
Study Program: Clinical Research; Vivian Chu, MD, Director  
Duke Institute for Health Innovation Clinical Research and Innovation Scholarship

10:30 – Julia Salinaro  
*Towards Reducing Operator Dependence in Fetal Ultrasound: Oriented 3D Volumetric Imaging of Second Trimester Fetuses and Reliability of Image Volume Interpretation by Expert and Novice Raters*  
Mentor: Josh Broder, MD  
Study Program: Biomedical Engineering and Surgery; Bruce Klitzman, PhD, Director
Biological Aging Drives Subtle Changes in the Host Genomic Response to Acute Respiratory Infection

Allison Bloom, Sunil Suchindran, PhD, Anna Mazur, Ephraim Tsalik, MD, PhD, Christopher Woods, MD, MPH, and Micah McClain, MD, PhD

Eugene A. Stead Student Research Scholarship

Background: The etiology of acute respiratory infections (ARIs) remains difficult to differentiate at the point of care despite modern microbiological techniques. There is growing interest in host biomarker assays, including those based on gene expression patterns in circulating cells, to aid in differentiation of viral and bacterial causes of ARI symptoms. However, there are concerns about how such tests perform in aging populations where host responses are often muted.

Methods: In order to assess the effects of aging on performance of gene expression-based biomarkers, we enrolled patients presenting to the ED with clinical ARI and selected 174 individuals aged </=25 and </= 60 years old with proven viral or bacterial ARI. Gene expression in peripheral blood was measured with Affymetrix microarrays. Published viral and bacterial signatures were applied to the data and Bayesian approaches were used to develop novel discriminative models. DAVID Bioinformatics Database was used to identify biological pathways which function differently in elderly individuals during viral and bacterial infections.

Results: We noted a marked decline in gene signature diagnostic performance between younger and older individuals in both viral (AUC 0.90 v 0.64) and bacterial (AUC 0.91 v 0.50) infections. This change in performance with age was driven by an overall more muted response in aging subjects that constrained the ability of genomic signatures to differentiate pathogen class. Furthermore, when examining the genomic differences driving the decline in signature performance, we found marked perturbations in expression of immunoglobulin genes and pathways driving known immunoregulatory mechanisms that provide novel insights into an age-related decline in ARI-focused immunity. Additionally, these genomic findings suggest a mechanism of immune exhaustion following viral infection that may drive some of the increase in post-viral bacterial superinfections seen in aging hosts.

Conclusions: Host-based, pathogen class-specific gene expression signatures offer great promise as diagnostic tools. However, altered immune responses in aging individuals are manifested at the genomic level and can affect diagnostic signature performance. For the first time we have demonstrated the critical importance of considering age during genomic biomarker development and application. Furthermore, studies of age-related differences in biomarker performance can lead to important breakthroughs in our understanding of age-associated alterations in immunity, evidenced here as we provide insight into a novel mechanism behind increased susceptibility to bacterial superinfection among the elderly.
The Taste of Calories in the Gut

Kelly L. Buchanan, Melanie M. Kaelberer, Marguerita E. Klein, Marcia M. Gomez, and Diego V. Bohórquez

HHMI Medical Research Fellowship

Background: Altered perception of sensory stimuli underlies disease states including obesity. Obesity is commonly linked to consumption of carbohydrates, likely because sugars exert powerful rewarding effects from both oral and post-ingestive pathways. But despite a similar gustatory sweet taste, the rewarding effects of artificial sweeteners are not as pronounced. Therefore, sucrose and sucralose must elicit differing responses in the gastrointestinal tract. A sensory epithelial cell that senses sugars in the gut is the enteroendocrine cell. Though classically studied from an endocrine perspective, we recently discovered that enteroendocrine cells synapse with vagal neurons and transduce sugar stimuli in vitro. We call these cells neuropods. Thus, we hypothesized that neuropods synapse with the vagus nerve to communicate the value of sugars and non-caloric sugar substitutes from gut to brain.

Methods: To test the functionality of this gut-brain neuroepithelial circuit, we performed whole nerve electrophysiology of the cervical vagus nerve. In anesthetized mice, we isolated and recorded from the right cervical vagus nerve. Nutritive sucrose and non-nutritive sucralose were infused into the proximal small intestine and vagal response was recorded. To specifically activate neuropods, we used a mouse with the light activated channel channelrhodopsin2 in CCK neuropods (CckCRE_ChR2). To determine the necessity of neuropods in this circuit, we used a mouse with the inhibitory light-activated anion channel halorhodopsin in CCK neuropods (CckCRE_Halo). We then investigated the receptors involved by inhibiting SGLT1 and sweet taste receptors.

Results: When delivered into the lumen of the intestine, both sucrose and sucralose significantly stimulate vagal firing rate. Using the specificity of optogenetics in CckCRE_ChR2 mice, we found that a 473nm intraluminal light stimulus rapidly and significantly increases vagal firing rate. In CckCRE_Halo mice, both the sucrose and sucralose vagal responses are attenuated by a 532nm light stimulus. Though both compounds activate sweet taste receptors, only the vagal response to sucralose, but not sucrose, is attenuated when murine sweet taste receptors are blocked with gurmarin. The opposite occurs when SGLT1 receptors are blocked with phloridzin.

Conclusions: The rewarding feeling of sugars begins in the gut. Neuropods not only form synapses with vagal nodose neurons, but are also necessary and sufficient to transduce senses from both sucrose and sucralose onto the vagus nerve. We show a vagally mediated pathway from gut to brain that can be stimulated by activation of different neuropod receptors. Synaptic communication provides the timing and specificity required to drive reward. This neuroepithelial circuit represents a therapeutic target to alter the transduction of caloric reward from gut to brain and to modulate appetite.
Innate Antiviral Immunity is Activated Upon Skin Injury Via an IL-27/STAT1 Axis

Jeffery Kwock, Chelsea Handfield, Jutamas Suwanpradid, Peter Hoang, Lauren Pontius, Amanda MacLeod

The Eugene A. Stead Jr. MD Research Scholarship

Background: Activation of antimicrobial immunity is critical to control infection following skin injury. Introduction of pathogens such as bacteria, viruses, and fungi to the wound area leads to the production of a variety of antimicrobial proteins. Antibacterial proteins, for instance, directly kill bacteria and are capable of facilitating wound repair. While the induction of antibacterial host defense molecules is relatively well-defined, regulation of antiviral proteins (AVPs) in the skin is largely unknown. A previous study in our lab suggested that interleukin-27 (IL-27) was capable of enhancing skin wound closure and inducing antiviral competence. We set out to determine whether AVPs are produced upon wounding, and if IL-27 is required for AVP production.

Methods: Expression of antiviral proteins upon wounding was measured by using human skin organ cultures and murine wound models. IL-27 was added to human keratinocytes in vitro and injected subcutaneously into murine ears in vivo. Human keratinocytes were pretreated with IL-27 and viral titering was used to measure change in viral infection by Zika virus. To determine whether IL-27 was required for wound-mediated production of AVPs, mice were wounded and neutralizing α-IL-27 antibody was applied to the wound bed. AVP expression in wounded Il27ra knockout (KO) mice was also compared to AVP expression in wounded wildtype (WT) mice. To determine mechanistically how IL-27 generates AVPs, we performed an siRNA screen in human keratinocytes stimulated with IL-27. We also measured nuclear translocation of STAT1 and IRF3 in human keratinocytes stimulated with IL-27. Lastly we examined how CRISPR-Cas9 KO of STAT1, STAT2, and TYK2 affected AVP expression in response to IL-27 in human keratinocytes.

Results: Skin wounding triggered the production of multiple AVPs, including OAS1, OAS2, and OASL (p < 0.05). As predicted, IL-27 induced OAS2 both in vitro and in vivo (p < 0.01) in a dose- and time-dependent manner. Addition of α-IL-27 neutralizing antibody to wounds or genetic knockout of the IL-27 receptor significantly decreased Oas2 (p < 0.01) suggesting that IL-27 plays a critical role in injury-induced production of OAS2. IL-27 was shown to protect keratinocytes from Zika virus infection in a cell-intrinsic manner (p < 0.01). siRNA screening revealed that OAS2 upregulation in response to IL-27 was IFNAR1-, TLR3-, STAT2-, MAVS-, and RIGI-independent, but STAT1- and IRF3-dependent (p < 0.001). In support of this pathway, IL-27 was shown to cause rapid and transient nuclear translocation of p-STAT1 and IRF3, and IL-27 failed to induce OAS2 in a STAT1KO keratinocyte cell line.

Conclusions: Together, our data suggest that IL-27 promotes skin antiviral competence through STAT1-dependent activation of AVPs upon cutaneous injury. New knowledge of the regulatory pathways of AVP induction following injury will aid in our understanding of cutaneous innate immunity and skin repair, potentially opening up avenues of research in protecting patients from skin-tropic viruses.
Extended HPV Genotyping: Comparing HPV Type-Distribution in Self and Provider-collected Samples

Claire Edelman, Dr. John Schmitt, Busola Sanusi, Dr. Lahoma Romocki, Dr. Jijay Sivaraman, Dr. Andrea Knittel, Dr. Lisa Rahangdale, Dr. Jennifer Smith

Background: Barriers to cervical cancer screening and prevention could be lessened with the addition of self-testing for high-risk HPV (hr-HPV). In addition, recent evidence suggests screening for specific hr-HPV genotypes should consider population differences among the subtypes most predictive of cervical intraepithelial neoplasia grade 2 or higher (CIN2+). Comparing hr-HPV subtype distributions between self and provider-collected samples, we evaluate the potential use of extended hr-HPV genotyping of self-swab samples to improve the reliability of primary hr-HPV screening.

Methods: 318 non-pregnant patients were enrolled, aged 25 to 65 years, who attended colposcopy clinic at UNC or Duke between November 2016 and March 2018 for abnormal screening or treatment for high grade dysplasia (CIN2+). Additionally, 43 participants with specifically normal cytology and positive hr-HPV for subtypes other than HPV 16 and 18 were recruited. Each participant collected a cervico-vaginal sample, followed by a provider-collected cervical sample prior to the colposcopy examination. Participants then completed a questionnaire. The hr-HPV subtypes found in each sample were detected using the BD Onclarity HPV assay. The primary outcome measured was concordance between provider and self-collected HPV DNA specimens. Additionally, multiple hr-HPV testing algorithms were evaluated for reliability of CIN2+ detection.

Results: A total of 260 patients met inclusion criteria. There was no significant difference (p > .05) between the overall frequency of self and provider hr-HPV positive results. When broken down by individual hr-HPV subtypes, there was no significant difference between provider and self-testing among biopsy proven lesions. The 3 most common HPV subtype groupings found in self-collected samples with CIN2+ lesions were, 16 (39.5%), 56/59/66 (16.3%), and 35/39/68 (15.1%). Using provider-collected samples as the gold standard, the overall sensitivity and specificity of self-collection was 84.2% and 75.8%. The overall percent agreement among the two tests was 81.2% with a Cohen’s kappa of .60. When restricted to only CIN2+ cases, the sensitivity, specificity, percent agreement, and Cohen’s kappa were 84.6%, 50.0%, 81.4%, and .24 respectively. Of the 86 cases of ≥CIN2+, 37 (43.0%) were either HPV 16 or 18 positive with both the self and provider test, while 50 (58.1%) were positive with the self-test and 51 (59.3%) were positive with the provider test for either HPV 16, (56/59/66), or (35/39/68). Finally, there was no significant difference between the frequency of individual hr-HPV subtypes among self and provider-collected samples.

Conclusions: Self-testing for hr-HPV has the potential to improve access to cervical cancer screening that is as reliable as provider-collected screening. The distribution of hr-HPV subtypes found among CIN2+ lesions is variable and future studies are necessary to determine the significance of this in guiding future genotype screening strategies as well as well as the makeup of future HPV vaccines.
Examining the Validity of Computerized Movement Tracking as an Index of Symptom Severity for Autism Clinical Trials

Andrew Yuan, Maura Sabatos-DeVito, Valerie Smith, Jill Lorenzi, Megan McVea, Brianna Herold, Michael Murias, Geraldine Dawson

Background: Core features of autism spectrum disorder (ASD) include deficits in social communication and restricted, repetitive patterns of behavior, interests, or activities. Current methods of behavioral assessment of ASD require extensive clinical assessments that can be time consuming and require subjective ratings by highly trained personnel. Scalable, quantitative, and objective methods for assessing behavior are critical for large scale clinical trials. Only one previous study has examined the use of computerized video tracking of movements in children with ASD and found that analysis of movement data in a parent-child interaction task (PCI) could provide unbiased and valid markers of severity of ASD. In this study, we further explore the utility of computerized movement tracking in a PCI task as a measure of ASD symptoms and functional skills of young children with ASD.

Methods: We analyzed movement data collected via computerized video tracking from 165 children with ASD (27-97 months old). The PCI task consisted of a six-minute session in a standardized room. A standardized set of toys was placed in the center of the room and at a table in the corner of the room and the child was told to play. The caregiver sat and remained in a chair in the corner opposite of the table. PCI video recordings were imported into Noldus EthoVision XT, a video tracking system previously used in animal studies that records and quantifies movement of participants in a naturalistic social environment. Within the software, the track of the child’s location data over time was analyzed with respect to four regions of interest (ROI) (center, table, caregiver, and periphery). Dependent variables included percent time spent in each ROI, latency to approach each ROI, and average distance between the child and caregiver. We examined associations of these video tracking measures with clinical assessments and parent report measures of core ASD symptoms and functional skills. Clinical measures were also used in statistical models as predictors of video tracking outcomes.

Results: Children’s movement patterns detected via computerized video tracking from the PCI were associated with severity of ASD symptoms and level of functional skills as measured by clinical assessment and parent report. Across multiple scales measuring cognition, language, and social communication, higher functioning children spent more time in the center region with toys and less time near their caregiver, near a table with toys in the corner of the room, and in the periphery of the room. Children were also slower to approach regions where they spent less time. Statistical modeling using clinical measures as predictors of video tracking outcomes demonstrated that the joint predictive ability of age, cognition, social skills, language, executive function, hyperactivity, and overall severity was significantly stronger than any one clinical measure alone.

Conclusions: These results suggest that measures of movement via video tracking may reliably quantify an overall impression of severity of ASD and functional ability. Additional research with this measure may support the use of video tracking as a scalable, quantitative, and objective assessment tool in the clinic or in clinical trials.
Adipose Stem Cell Crosstalk with Chemo-residual Breast Cancer Cells: Implications for Tumor Recurrence

Matthew A. Lyes, Sturgis Payne, Paul Ferrell, Salvatore V. Pizzo, Scott T. Hollenbeck, Robin E. Bachelder
The Donald B. Hackel Fellowship

Background:
Most triple-negative breast cancer (TNBC) patients exhibit an incomplete response to neoadjuvant chemotherapy, resulting in chemo-residual tumor cells that drive tumor recurrence and patient mortality. Accordingly, strategies for eliminating chemo-residual tumor cells are urgently needed. Although stromal cells contribute to tumor cell invasion, to date, their ability to influence chemo-residual tumor cell behavior has not been examined. Our study is the first to investigate crosstalk between adipose-derived stem cells (ASCs) and chemo-residual TNBC cells. We examine if ASCs promote chemo-residual tumor cell proliferation, having implications for tumor recurrence.

Methods:
ASC migration toward chemo-residual TNBC cells was tested in a transwell migration assay. Importance of the SDF-1/CXCR4 axis was determined using neutralizing antibodies and a small molecule inhibitor. The ability of ASCs to drive tumor cell proliferation was analyzed by culturing tumor cells +/- ASC conditioned media (CM) and determining cell counts. Downstream signaling pathways activated in chemo-residual tumor cells following their exposure to ASC CM were studied by immunoblotting. Importance of FGF2 in promoting proliferation was assessed using an FGF2-neutralizing antibody.

Results:
ASCs migrated toward chemo-residual TNBC cells in a CXCR4/SDF-1-dependent manner. Moreover, ASC CM increased chemo-residual tumor cell proliferation and activity of extracellular signal-regulated kinase (ERK). An FGF2-neutralizing antibody inhibited ASC induced chemo-residual tumor cell proliferation.

Conclusions:
ASCs migrate toward chemo-residual TNBC cells via SDF-1/CXCR4 signaling, and drive chemo-residual tumor cell proliferation in a paracrine manner by secreting FGF2 and activating ERK. This paracrine signaling can potentially be targeted to prevent tumor recurrence.
Ketamine versus Midazolam for Prehospital Agitation

SK Scharber, GB Horton, LR Klein, BE Diver, TD Olives, JC Moore, JB Cole

Background: Agitated patients in the prehospital environment often pose a threat to themselves or others and can impede transport or facilitation of their own emergent medical care. Prolonged agitation has the potential to lead to physical exhaustion, lactic acidosis and even death. Controversy exists regarding the ideal sedation protocol for agitated patients in the prehospital environment. We hypothesized a ketamine-based tiered dosing protocol would be superior to a midazolam-based tiered dosing protocol for prehospital agitation.

Methods: This is a prospective open label study of all adults in our EMS system transported to our ED requiring treatment for agitation. All paramedics were trained in the Altered Mental Status Scale (AMSS), a validated ordinal scale of agitation from -4 (coma) to 0 (normal) to +4 (most agitated). From August 2017 to January 2018, all patients were treated with protocolized tiered doses of intramuscular (IM) ketamine, based on AMSS: 3 mg/kg for severe agitation (AMSS +2/+3) and 5 mg/kg for profound agitation (AMSS +4). From February 2018- August 2018, the protocol changed to tiered doses of IM midazolam: 5 mg for severe agitation and 15 mg for profound agitation. Paramedics carried stopwatches and measured time to adequate sedation (TAS), our primary outcome. TAS was analyzed using differences in medians and 95% confidence intervals (CIs). Secondary outcomes included safety events.

Results: 306 subjects have thus far been enrolled; 203 received ketamine, 103 received midazolam. Median age was 33 (range 18-75); 69% were male. A contributing etiology of agitation was alcohol in 209 (68%) cases (median alcohol concentration 210 mg/dL), and suspected drug intoxication in 120 (39%). Regarding the primary outcome, for all patients receiving ketamine median TAS was 4.3 minutes (range 1-73.6) vs. 7.9 minutes (range 0.5-71.5) for midazolam (diff -2.8 mins, 95% CI -4.4 to -1.7). For severe agitation, median TAS for ketamine was 4 minutes (range 1-73.6) vs. 6.8 minutes (range 0.5-64.5) for midazolam (diff -2.0 mins, 95% CI -3.8 to -0.5). For those with profound agitation, median TAS for ketamine was 4.6 min (range 1.2-27.3) vs. 8.8 min (range 1.5-71.5) for midazolam (diff -3.9 mins, 95% CI -6.4 to -2.0). A Kaplan Meier survival function was performed on TAS (Figure 1). For ketamine, 42 (21%) required additional prehospital sedation vs. 14 (14%) for midazolam (diff 7%, 95% CI -2% to 16%). Safety results were as follows: intubation (ketamine 25%, midazolam 20%, diff = 5%, 95% CI -5% to 15%), apnea (19%, 19%, 0%, -10% to 9%), hypersalivation (23%, 2%, 21%, 15% to 28%), and vomiting (6%, 1%, 5%, 2% to 9%).

Conclusions: For prehospital agitation, a ketamine based tiered dosing protocol resulted in a shorter TAS compared to midazolam. The difference in TAS was larger in the most profoundly agitated patients, suggesting ketamine may be the superior treatment for profound agitation and possibly prevent the complications of prolonged agitation.
Novel Nanoparticle Formulation of Niclosamide Treats Metastatic Human and Canine Osteosarcoma in vivo

Gireesh B. Reddy, BS, David L. Kerr, MD, Artak Tovmasyan, PhD, Prasad Walke, PhD, David Hsu, MD, PhD, Jason A. Somarelli PhD, David Needham PhD, William C. Eward, DVM, MD

Background: Osteosarcoma (OS) is the most common primary bone malignancy and the third most common cancer of childhood. Unfortunately, it has seen relatively few therapeutic advances in the past three decades with minimal improvements in both survival amongst patients presenting with metastatic disease and treatment-derived morbidity resulting from established chemotherapeutics. The anthelminthic drug niclosamide has recently demonstrated inhibitory effects on several pathways known to be dysregulated in OS. However, the drug’s extremely low bioavailability and need for solubilizing agents have hampered niclosamide’s therapeutic response. To overcome this challenge, we have developed a novel niclosamide stearate prodrug therapeutic (NSPT), made of a lipid nanoparticle formulation of esterified niclosamide. Further establishing the efficacy and survival benefits of NSPTs will be crucial for clinical translation of this work with the ultimate goals of improving survival and decreasing morbidity for human and canine OS patients.

Methods: Nanoparticles were prepared by solvent-solvent exchange of niclosamide stearate dissolved in acetone with water in a 1:9 v/v ratio and then concentrated using centrifugal filtration. Dose-response assays and western blot analyses were performed on cultured canine and human OS cells after treatment with NSPTs. The OS cells were transduced with lentiviruses encoding firefly luciferase, NLS mCherry, and cytoplasmic zsGreen. NSPT effects on OS proliferation and apoptosis cells were studied in vitro using live-cell multiplexed imaging and ex vivo using a lung explant pulmonary metastasis assay. Mouse models of osteosarcoma metastasis were generated via tail-vein injection of 1x10^6 luciferase-labeled D418 OS cells into 6-week-old SCID/beige mice. Mice were randomized to PBS, NSPT (50 mg/kg i.v.), or doxorubicin (1.2 mg/kg i.p). Bioluminescence in average radiance (p/s/cm^2/sr) was measured as an indicator of intravital tumor burden.

Results: Dose-response assays demonstrated that NSPTs inhibited canine OS cell growth in vitro. Western blot analysis showed reductions in phospho-S6, with cell line-specific reductions of phospho-STAT3, phospho-Akt, and mTOR. Ex vivo, NSPT treatment groups significantly reduced lung tumor burden at 5 days vs. PBS. In vivo, mice treated with NSPTs had significantly lower overall lung tumor burden. No sequelae of NSPT therapy were observed at this higher dosage, while doxorubicin treated mice lost weight during treatment.

Conclusions: NSPTs are effective chemotherapeutic agents in metastatic OS in vivo, leading to decreased metastatic tumor burden in both dog and human OS. NSPTs are able to modulate antitumor effects without the treatment-derived morbidity of standard-of-care chemotherapeutics, such as doxorubicin. This chemotherapy strategy has significant potential to shift the clinical framework of osteosarcoma management and to improve survival for patients with osteosarcoma, while the similarities in response between canine and human OS provide a unique opportunity to develop NPSTs clinically in the veterinary space in order to accelerate development in the human setting.
Advance Care Planning and Quality of Death: Perceptions in Northern Tanzania

Temitope Gafaar, Catherine Staton, Oliver Henke, Joao Ricardo Vissoci, Blandina Mmbaga

Doris Duke International Clinical Research Fellows Program

Background: Global interest in end-of-life (EoL) care is increasing. Due to advancements in modern medicine, particularly intensive care treatments, the process of dying is happening over longer periods of time for many individuals. Furthermore, the world’s population is aging and the annual death rate is set to continue increasing. Since the 1990s, advance care planning (ACP) has been promoted in high-income countries and has been associated with increased quality of life for patients and their families, and higher satisfaction with quality of care. However, in sub-Saharan Africa - where the disease burden is increasingly augmented by non-communicable diseases – ACP protocols are not common. At the Kilimanjaro Christian Medical Center (KCMC) in Moshi, Tanzania, there are no advance directive (AD) tools being used to document patient preferences for EoL care. There is a general avoidance of the topic and the issue of introducing ADs in healthcare-limited regions can be an ethically complex subject. Nonetheless, evidence from similar settings indicate that an appropriate quality of life (QoL) is valued, even as one is dying. What differs amongst cultures is their definition of a ‘good death’ and the impact of advance EoL planning on the quality of death. The goal of this study was to qualitatively evaluate perceptions of the quality of death and attitude towards ACP amongst certain groups of people who have been confronted with the concept of EoL – patients, their relatives/friends, and healthcare workers – in Tanzania.

Methods: A total of 122 participants for 13 focus group discussions. FGDs were conducted in Swahili using a semi-structured guide. These discussions were audio-recorded, transcribed, translated, and coded using an inductive approach.

Results: Nearly all participants believed that death could be qualified into ‘good’ and ‘bad’. Even though participants expressed belief that death and its timing were ultimately in God’s hands, a major theme of what qualified as good death was ‘Being Prepared or Having Planning Ahead’. Emphasis was placed on its multidimensionality i.e. spiritual/religious, interpersonal/familial relationship, mental health, and financial well-being. Conversely, a ‘bad death’ was associated with ‘Being Unprepared or Lack of Preparation’. Non-Preparation-associated themes that further qualified death were adequacy of comfort and healthcare adequacy/quality and the magnitude of disease burden.

Conclusion: In Northern Tanzania, most of the major determinants of how people judge ‘Good vs Bad death’ can be categorized into: ‘how well one has lived with God and with others’ and ‘how well one has left his/her affairs and family behind’. Furthermore, participant responses indicated a trend towards accepting and favoring advance planning for EoL and its documentation. Based on the results, it was found that introducing an AD that emphasizes spiritual or religious well-being, interpersonal/family relationships, and QoL desires may be culturally appropriate at KCMC.
Deeply-Personalized Medicine: Bringing Deep Learning to Sepsis Care


Duke Institute for Health Innovation Clinical Research and Innovation Scholarship

**Background:** Clinical decision support tools based on predictive analytics can provide actionable information and improve clinical outcomes for patients at risk of developing sepsis. Scoring systems such as Systemic Inflammatory Response Syndrome (SIRS), National Early Warning Score (NEWS), quick Sepsis Related Organ Failure Assessment (qSOFA) tend to lack specificity, leading to high false alarm rates and physician alarm fatigue. Our academic institution previously implemented NEWS to prompt assessment for sepsis, but 63% of alarms were cancelled by the care nurse. The goal of this study was to develop a machine learning model to improve early detection of sepsis, leveraging state-of-the-art deep learning.

**Methods:** This was a retrospective study of all adult inpatient admissions (n=43,046) at a large academic hospital from October 1, 2014 to December 31, 2015. Patient demographics, comorbidities, vitals, lab results, and medication data were extracted from the electronic health record (EHR), yielding 86 predictor variables. Our dataset included 25 million vital sign measurements, 1.9 million medication administrations, and 5.2 million lab results. We used deep recurrent neural networks (RNNs), a form of deep learning for sequential data, to predict onset of sepsis. We combined this deep learning model with multi-output Gaussian processes (MGPs), which interpolate and impute continuous functions for physiological time series variables, to create our final model, denoted MGP-RNN. We compared MGP-RNN with random forest (RF), Cox regression (CR), penalized logistic regression (PLR), SIRS, NEWS, and qSOFA. We calculated C-statistics and false alarms per true alarm at 80% sensitivity, as a function of the number of hours in advance of sepsis.

**Results:** The C-statistic at 80% sensitivity for predicting 4 hours before sepsis was 0.89 for our MGP-RNN compared to 0.83 for RF, 0.82 for CR, 0.79 for PLR, 0.66 for SIRS, 0.60 for NEWS, and 0.57 for qSOFA. Even 12 hours prior to sepsis, the MGP-RNN maintained a C-statistic of 0.87. At an average number of 3 alarms raised per hour, our model was able to detect 10.1 sepsis cases per day whereas SIRS, NEWS, and qSOFA were only able to detect 5.9, 3.0, and 2.2 sepsis cases per day, respectively. Using our model, sepsis was detected at a median time of 5 hours prior to clinical definition of sepsis.

**Conclusions:** In this study, we developed a deep learning approach to improve early detection of sepsis. Our model consistently outperformed strong baseline methods and had considerably fewer false alarms than traditional clinical risk scores used to detect sepsis today. We have developed a framework to integrate our MGP-RNN into clinical practice and will begin a phased implementation of this analytics-augmented clinical workflow in our academic institution in the upcoming months. Our technical infrastructure can be scaled to other sites that use similar EHRs and our model framework can be extended to other inpatient complications such as cardiogenic shock, cardiopulmonary arrest, and inpatient mortality.
Towards Reducing Operator Dependence in Fetal Ultrasound: Oriented 3D Volumetric Imaging of Second Trimester Fetuses and Reliability of Image Volume Interpretation by Expert and Novice Raters

Julia R. Salinaro, BA; Patricia McNally, RDMS; Joao Nickenig Vissoci, PhD; Sarah Ellestad, MD; Brian Nelson, MD; Joshua Broder, MD

**Background:** Fetal ultrasound (US) is inherently operator dependent; sonographers must be highly trained to perform scans and capture appropriate images. We developed a low cost system enabling acquisition of oriented, three-dimensional (3D) image volumes using any existing 2DUS machine. We hypothesized that a novice sonographer could use this system to acquire image volumes of second trimester fetuses that could then be interpreted for assessment of basic parameters and reliable biometric measurements by novice and expert raters.

**Methods:** A novice sonographer swept the 2DUS transducer, paired with the research system, across the region of interest to acquire image volumes of second trimester fetuses from 32 subjects. Volumes were reconstructed using a pixel-based algorithm. Acquisition and reconstruction times were automatically recorded. Image volumes were viewed in 3DSlicer; the percentage of interpretable volumes was recorded. An expert made blinded, timed assessments of placental location (PL), fetal presentation (FP), and amniotic fluid volume (AFV) for each subject. Both novice and expert raters blindly, independently measured biparietal diameter (BPD), humerus length, and femur length; corresponding gestational age (GA) estimates were calculated. Inter-rater reliability of measurements and associated GAs between each rater and clinical US reports were assessed by intraclass correlation coefficient (ICC). Mean inter-rater measurement differences were analyzed by one-way ANOVA.

**Results:** Volume acquisition and reconstruction required mean 30.4s (±5.7) and 70.0s (±24.0), respectively. PL, FP, and AFV could be evaluated from volumes for all subjects. At least one biometric measurement was possible for 31 subjects (97%). Mean time for expert assessment of PL, FP, and AFV was 16s (±0.0). Agreement for all measures between all rater pairs was excellent (ICCs ≥0.95). ICCs between all rater pairs for each structure and corresponding GA estimate demonstrated either good or excellent reliability. ICCs were highest for GA estimates by BPD between both raters and reported values (expert ICC: 0.97 CI95% 0.94-0.99; novice ICC: 0.97 CI95% 0.94-0.99). Mean inter-rater differences were not significant.

**Conclusions:** Comprehensive, oriented image volumes of second trimester fetuses were rapidly acquired by a novice and reconstructed for interactive visualization. PL, FP, and AFV could be quickly assessed by an expert for all subjects. Measurements of BPD, femur, and humerus could be made for the vast majority of subjects. Both the measurements and associated GAs were highly reliable between both novice and expert raters and clinical reports. This low cost technology has the potential to reduce the operator dependence of US, which could facilitate implementation of obstetric US in low resource settings where expert sonographers may be unavailable and task shifting in areas with established US use.
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Evaluation of Diagnostic Criteria for Hashimoto’s Encephalopathy in Children and Adolescents

Ashley V. Adams, BA, William Gallentine, DO, Heather Van Mater, MD, GenaLynne Mooneyham, MD

Background: Hashimoto’s Encephalopathy (HE) refers to a state of encephalopathy associated with anti-thyroid antibodies that is commonly responsive to steroids. The majority of adult patients treated for HE meet the requirement for subclinical or mild thyroid disease as part of the recently proposed adult diagnostic criteria for the disease. However, while HE in children has been described in limited capacity, the majority of case reports indicate that most children treated for HE do not have evidence of thyroid disease. Further, while the current criteria necessitate hallucinations, seizures, myoclonus, or stroke-like symptoms, there is no data on the prevalence of these specific symptoms in pediatric cases of HE. We aim to describe our experience with HE and evaluate the impact of applying these current diagnostic criteria to pediatric patients.

Methods: A total of 18 patients ≤ 18 years old were treated for HE through the Duke University Autoimmune Brain Disorders Program from 2014-2017 based on the abrupt onset of neuropsychiatric symptoms in the presence of high titer anti-thyroid antibodies after exclusion of alternative diagnosis. Patients were evaluated at time of symptom onset and follow up ≥ 1 year after initiation of immunomodulatory treatment for degree of impairment within the neuropsychiatric domains of cognition, language, psychiatric disturbance, seizure, movement disorder, sleep disruption, and overall functionality. We compared the response to treatment among patients stratified by the presence or absence of a) subclinical or mild thyroid disease, and/or b) hallucinations, seizures, myoclonus, or stroke-like symptoms.

Results: Of these 18 pediatric patients, 6 met full adult diagnostic criteria while 11 patients did not meet criteria solely due to the absence of thyroid disease. 67% of the 6 patients who met full adult criteria and 82% of the 11 patients who did not meet full criteria based on their absence of thyroid disease demonstrated moderate to substantial improvement in overall functionality at follow up. At symptom onset, 100% of these combined 17 patients exhibited moderate or severe cognitive impairment paired with deficits in ≥ 3 other neuropsychiatric domains. 76% of these 17 patients presented with severe psychiatric impairment. One patient did not meet either criterion of thyroid disease or hallucinations, seizures, myoclonus, or stroke-like symptoms, and improved from severe impairment back to baseline with treatment.

Conclusions: Rigidly applying the current diagnostic criteria to pediatric patients with suspected HE might result in the failure to treat potential responders. We propose a broader set of diagnostic criteria for HE in children that 1) does not require thyroid disease, 2) includes abrupt onset cognitive regression with deficits in ≥ 1 other neuropsychiatric domain(s), and 3) includes the presence of high titer anti-thyroid antibodies.
Mixed-Methods Assessment of HVTN 704/HPTN 085 Participants’ Experience with the Pre-Exposure Prophylaxis (PrEP) Referral Program

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Background: HVTN 704/HPTN 085, also known as “AMP” is a Phase 2b clinical trial which evaluates the efficacy of a broadly-neutralizing monoclonal antibody, VRC01, in reducing HIV-1 infection among men and transgender individuals who have sex with men (MSM). As an incentive to participants, the AMP Pre-Exposure Prophylaxis (PrEP) Referral Program links willing AMP participants to a participating provider. In some cases, it covers a portion of the costs associated with starting Truvada™ as PrEP, including prescription costs and required labs at 3-month follow-up visits. Given the increased burden of HIV infection in communities of color and low-socioeconomic status, we sought to create a narrative for the participant experience with the referral program and explore its role in mitigating this disparity.

Methods: Eligible participants were enrolled in the AMP Study (HVTN 704/HPTN 085) at the Columbia Research Unit and New York Blood Center (NYBC)- Project Achieve HIV Vaccine Trials Network (HVTN) study sites in New York City. Participants were recruited using online flyers, e-mail advertisements, and word-of-mouth via study staff and clinicians. Informed consent was obtained from each participant. A ~30-minute, semi-structured, in-depth interview and a 42-item, online HIV risk and PrEP usage questionnaire were administered to each participant. Interviews were digitally-recorded, transcribed, and analyzed for themes in ATLAS.ti Qualitative Data Analysis software. Questionnaire summary data were analyzed using the IBM SPSS statistical package.

Results: Thirty-nine participants were recruited (n=21 from Columbia Research Unit and n=18 from NYBC). Seventy percent (n=27) of study participants reported current PrEP use. Forty-four percent (n=12) of those individuals endorsed usage of the AMP PrEP Referral Program. PrEP uptake was lowest among black-identifying participants; they were also 20% less likely than their white-identifying counterparts to be taking it one year from now. Sixty-four percent (n=9) of individuals not taking PrEP reported a gross annual income of less than $40,000. An iterative qualitative analysis yielded 58 unique codes and over 2000 quotations. Major interview themes included 1) limited awareness of AMP PrEP Referral Program, 2) previous difficulty seeking PrEP outside study, 3) redundancy of linkage process to clinics that were unaffiliated with the study site (e.g. non-Columbia clinics), 4) limited participant understanding of cost and safety data supporting use of Truvada™ as PrEP, and 5) beliefs of a low-risk profile and self-efficacy among non-PrEP users.

Conclusions: Uptake of PrEP was limited among black individuals and low socioeconomic status in the study sample despite provisions made by the AMP PrEP Referral Program. Strategies for gauging participant HIV health beliefs, improving participant comprehension of AMP PrEP Referral Program financial incentives, and streamlining the provider linkage component may increase PrEP uptake within these high-risk groups. Future studies should be geared towards providing more robust data on the demographic-specific effectiveness and reach of the AMP PrEP Referral Program at other HVTN study sites.
Scheduled Postoperative Ripcord Removal in Baerveldt 350 Implants: A Prospective, Randomized Trial

Selena J. An, MSPH, MA, Joanne C. Wen, MD, Michael S. Quist, MD, Elizabeth W. Mathenge, MD, MSc, Anita Vin, MD, Leon W. Herndon, Jr., MD

Background: Many surgeons remove the ripcord in the Baerveldt (BVT) glaucoma drainage device to better control tube opening and intraocular pressure (IOP) lowering postoperatively. However, complications following BVT implant surgery with or without ripcord removal are not well characterized. We performed a prospective, randomized trial to test the hypothesis that scheduled ripcord removal decreases complications and final IOP.

Methods: Eighty-one patients were enrolled and randomized to scheduled ripcord removal at postoperative week 3 or to observation. They were followed for 6 months, and outcomes were compared between the two groups.

Results: Forty-four patients were randomized to scheduled ripcord removal and 37 to observation. The removal group had higher rates of total complications (66% versus 41%, p=0.022), a lower rate of tube fibrin obstruction (2.3% versus 16%, p=0.026), and a larger decrease in the number of medications (1.3 versus 0.5 medications, p=0.034). The removal group’s mean IOP decrease was 8.6mmHg and success rate was 52%, defined as 5mmHg<final IOP≤15mmHg without further surgical intervention. Neither differed significantly from those of the control group.

Conclusions: This study suggests that scheduled ripcord removal is correlated with more complicated recovery in comparison to spontaneous tube opening, though the complications were largely self-limited and not sight-threatening. This technique leads to similar IOP decrease and success rate and is also associated with a greater decrease in the number of medications, which may improve quality of life for patients. Additional studies could be useful in clarifying this technique’s role in the postoperative course among different patient populations.
Latent Class Mixed Modeling of Serum Creatinine Trajectories: A Novel Approach to Assessing Acute Kidney Injury Following Cardiac Surgery

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Supported by the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number TL1TR001116

Background: Acute kidney injury following cardiac surgery is a frequent and serious complication. Despite this, little progress has been made in developing either preventative or therapeutic interventions. This may be, in part, due to the heterogeneous nature of both the pathophysiology and classification schema of acute kidney injury itself. Consensus diagnostic and severity criteria utilize simple creatinine thresholds that ignore potentially valuable information contained within the postoperative creatinine trajectory (i.e., pattern of change over time). Patients with similar trajectories may represent previously unrecognized acute kidney injury phenotypes with important pathophysiologic and clinical implications.

Methods: A retrospective cohort study of adult patients undergoing isolated, non-emergent coronary artery bypass grafting from 1/1/2000 – 12/31/2009 was conducted using a development-validation approach. Latent class mixed modeling was utilized to identify, in an unbiased manner, unique latent classes of patients with distinct serum creatinine trajectories over the first four postoperative days. Association between perioperative characteristics was assessed with multinomial logistic regression. Survival patterns of the identified classes were examined using the Kaplan-Meier method. Classes with similar survival profiles were clustered into trajectory risk groups, and adjusted Cox proportional hazards regression was utilized to assess the association between risk group membership and mortality.

Results: In the development cohort (n = 2647), 12 latent trajectory classes were identified. Classes were heterogeneous with respect to rate, timing and level of creatinine rise (or fall) as well as the presence and completeness of recovery. Several perioperative characteristics were significantly associated with trajectory class membership. Survival varied across trajectory classes, and two distinct survival profiles representing low- and high-risk trajectories were identified. Membership within the high-risk group was significantly associated with long-term mortality (adjusted hazard ratio 2.30; 95% CI [1.60 – 3.32]). Validation testing (n = 2647) revealed 12 trajectory classes that were qualitatively similar to the development cohort, with comparable clusters of risk groups and resultant survival patterns.

Conclusions: In conclusion, we identified and validated 12 unique classes of patients with distinct serum creatinine trajectories following cardiac surgery. Membership in a high-risk trajectory group was independently associated with long-term risk for mortality. Using serum creatinine trajectories is a novel approach to characterizing importantly different acute kidney injury phenotypes. These phenotypes may be reflective of distinct endotypes of renal injury with key differences in genetic and pathophysiologic characteristics. Further study of these trajectory-based phenotypes to identify these differences may provide an alternative approach to subdividing patients for assessment of existing and future putative reno-protective strategies.
Effects of Pathogen Reduction on the Hemostatic Function of Cryoprecipitate

Ansari A, Kamyszek RW, Srinivasan AJ, Stoner K, Welsby IJ

Background: Despite rigorous blood safety measures, blood product transfusion continues to pose risks for transmission of emerging pathogens, bacteria, and protozoa. Pathogen reduction is a method of treating blood products to prevent transmission of these harmful agents. While pathogen reduced fresh frozen plasma (FFP) has been shown to have reduced coagulant factor activity using rotational thromboelastometry (ROTEM®; Instrumentation Laboratory, Bedford, MA), pathogen reduced cryoprecipitate (PR-cryo) has not been thoroughly evaluated in a similar manner. The purpose of this study was to utilize a ROTEM-EXTEM-based, in vitro model of dilutional coagulopathy to evaluate the effect of simulated PR-cryo vs non-PR-cryo transfusions on clot formation.

Methods: In this IRB-exempt study, an in vitro model of a coagulopathic patient was established by diluting pooled normal plasma with saline in a 1:1 ratio to target a maximum clot firmness (MCF) that would typically trigger transfusion of cryoprecipitate. This diluted plasma model was subsequently used to model the effect of cryoprecipitate added in a proportion simulating successive in vivo transfusions of ~100ml of cryoprecipitate. Apheresis derived (APH) and whole blood derived (WB) group A donor plasma was pooled and split to produce six identical APH and six WB cryoprecipitate units. Three APH and three WB units were subject to PR treatment according to manufacturer recommendations using amotosalen and UVA light (INTERCEPT® Blood System, Cerus Corporation, Concord, CA). The remaining three APH and three WB units served as non-PR-cryo controls. Three variables were measured using EXTEM: (1) clotting time (CT), (2) MCF, and (3) alpha angle. EXTEM variable dose responses to sequential simulated PR and non-PR-cryo transfusions were analyzed using Spearman correlation. Inter-group comparisons between PR and non-PR-cryo were performed using nonparametric pairwise Wilcoxon rank tests. Significant results were declared at p< 0.05. Statistical analyses were performed using JMP Pro 13 software.

Results: Successive PR-cryo doses were significantly correlated with an overall decrease in CT (Spearman ρ= -0.26, p = 0.042), and an increase in both MCF (ρ=0.79, p < 0.001), and alpha angle (ρ=0.71, p < 0.001). Addition of successive non-PR-cryo doses was also significantly correlated to an overall decrease in CT (ρ= -0.32, p = 0.011), and increase in both MCF (ρ=0.65, p < 0.001), and alpha angle (ρ=0.63, p < 0.001). Between treatment groups, PR-cryo and non-PR-cryo did not demonstrate significant differences in CT, MCF, or alpha angle at all three dose levels.

Conclusions: Our data from this study suggest that pathogen reduction, by means of the INTERCEPT Blood System, does not reduce the hemostatic effect of cryoprecipitate. Further quantitative studies to examine the potential effects of pathogen reduction on coagulation factor and protein content in cryoprecipitate are warranted.
Assessing Surgical Quality in a Low Resource Setting with a Novel Hospital Assessment Tool: A Pilot Study in Brazil

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Program in Global Surgery and Social Change

Background: Adverse events from surgical care are a major cause of death and disability in low- and middle-income countries (LMIC). Metrics for quality of surgical care in high income countries are resource intensive and inappropriate in most LMIC settings. A new tool to measure surgical quality in LMICs was recently developed. We conducted a pilot study to evaluate the feasibility of applying this tool in a resource-constrained setting.

Methods: The pilot was performed at a tertiary hospital in Amazonas, Brazil. The tool was adapted to the local context resulting in 14 metrics (Table 1). The presence of a morbidity and mortality conference, perioperative mortality rate, procedure density, and readmission rates were retrospectively extracted from the hospital administrative data and OR logbooks. The remaining metrics were collected prospectively during a 4-week period by external data collectors using an OR observation checklist and a patient discharge questionnaire adapted from validated tools.

Results: A total of 183 surgeries were observed and 125 patient questionnaires administered. Results are found in Table 1. Using the modified tool, 12 metrics were successfully collected, and 2 are still being processed.

Conclusions: It is feasible to apply this surgical quality measurement tool in resource-limited settings. With further applications of the tool in other low resource settings, the measures and targets can be refined and a weighting system developed to better guide surgical quality improvement measures at the facility, regional, and national levels.

Table 1: Modified Tool with Indicator Results

<table>
<thead>
<tr>
<th>DONABEDIAN FRAMEWORK</th>
<th>Structure</th>
<th>Process</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morbidity and Mortality Conference</td>
<td>No Conference</td>
<td>Safe Surgery Checklist use = 0%</td>
<td>Peri-operative mortality rate</td>
</tr>
<tr>
<td>Provider Density Proxy: Fully Qualified Surgeon Present 98%</td>
<td>Procedure Rate 710 procedures per 100,000 population</td>
<td>710 procedures per 100,000 population</td>
<td>Cesarean Rate Proxy: Readmission rates within 30 days Case Clavien Dindo&gt;2 Unable to Collect</td>
</tr>
<tr>
<td>-</td>
<td>Use of Consent 74%</td>
<td>-</td>
<td>Patient Hospital Satisfaction Questionnaire Summary Star Rating 4</td>
</tr>
<tr>
<td>Travel time to hospital Median = 4H Within 2H = 32%</td>
<td>Time from ED presentation to non-elective abdominal surgery Median = 8h</td>
<td>Daily OR Utilization 48%</td>
<td>Follow up plan 47%</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Patient Median Income to Catchment Population 1.2</td>
<td>-</td>
<td>-</td>
<td>Catastrophic patient reported expenditure 2.5%</td>
</tr>
</tbody>
</table>
Germline-Targeting and Reverse Engineering to Elicit the Ch235.12 Broadly Neutralizing Antibody Lineage

Shay Behrens, David C. Montefiori
National Institute of Allergy and Infectious Diseases (NIAID) U.S. Public Health Service
Grant UM1 AI068614, HVTN Research and Mentorship Program (RAMP)

Background: The ability to stimulate germline B cells that give rise to broadly neutralizing antibodies (bNAbs) is a major goal for HIV-1 vaccine development. bNAbs that target the CD4-binding site (CD4bs) exhibit extraordinary potency and breadth of neutralization and are particularly attractive to elicit with vaccines. Glycans that border the CD4bs and impede the binding of germline-reverted forms of CD4bs bNAbs are potential barriers to naïve B-cell receptor engagement. Targeted deletion of a subset of these glycans by sequon mutation has permitted binding but not neutralization, suggesting additional barriers exist. We hypothesized that small Man5 glycans replaced larger complex-type glycans reducing steric barriers to germline CD4bs bNAb binding without disrupting native Env conformation. Additionally, we hypothesized that specific HIV Env modifications would permit neutralization by germline-reverted CH235.12 UCA.

Methods: We used site-directed mutagenesis to create various CH0505TF mutants with the goal of identifying earlier precursor intermediates. HIV-1 mutants were then produced in cells lacking the enzyme N-acetylglucosaminyltransferase (GnTI-) to enrich for Man5 glycoforms of N-linked glycans that would otherwise be processed into complex-type glycans. We determined the neutralization tier phenotype of mutant viruses and used IC50 to determine neutralization capability and antibody breadth.

Results: HIV Env mutations in Loop D (N279K) and V5 (G458Y) in combination with Man5-enrichment rendered autologous CH0505TF Env highly sensitive to neutralization by CD4bs bNAb, CH235.12 UCA.

Conclusions: These findings advance our understanding of the restrictions imposed by glycans in the elicitation of CD4bs bNAbs and suggest a vaccine strategy to initiate and mature the CH235.12 lineage in addition to providing a standardized screening tool for early bNAb precursors in clinical trials.

Figure 1. Immunization strategy to elicit CH235.12-like bNAbs.
Revision Total Joint Arthroplasty: Final Stop Tertiary Referral Center

Michael Bergen BS, Sean Ryan MD, Cierra Hong BA, Michael Bolognesi MD, Thorsten Seyler MD PhD

Background: Prior changes to Medicare’s Diagnosis Related Groups (DRGs) were partially driven by evidence that revision total hip and knee arthroplasty (THA, TKA) had greater cost and complications than primary arthroplasty. Failure to adequately reimburse revision arthroplasty was unequally burdening referral centers. Tertiary centers would, ideally, function to manage patients with complex diseases or multiple comorbidities requiring medical co-management. We aimed to determine if modern pay structures have resulted in proper utilization of a tertiary referral center, with unhealthier patients being referred from greater distances.

Methods: All patients who underwent primary or revision TKA or THA at the investigating institution from 2012 to 2016 were identified using a comprehensive institutional database. Patient demographics, preoperative Charlson Comorbidity Index (CCI), operative data, and postoperative course were identified, and travel distance was calculated from each patient’s home address and stratified into <25 mi (local), 25-75 mi, and >75 mi (referral). Patients were analyzed based on procedure performed (primary/revision, hip/knee) and by distance from the investigating institution.

Results: 4,245 procedures were included for analysis, including 3,257 primary (53.9% hip) and 988 revision (56% hip) arthroplasties. Patients living >75 miles away had significantly higher odds of undergoing revision arthroplasty compared to patients living within 25 miles of the institution (knee: odds ratio (OR)=2.43; hip: OR=2.61; both p<.001). For both primary and revision patients, CCI did not increase with distance from the investigating institution. Patients traveling >75 mi were more likely to have periprosthetic fracture (OR=3.9; p=.011) and less likely to have dislocation (OR=0.54; p=.026) as the surgical indication for revision.

Conclusions: Ultimately, patients referred to a tertiary referral center were more likely to necessitate revision TJA; however, these patients did not differ in comorbidity status compared to local patients. Additionally, periprosthetic fracture, a particularly expensive and high-risk surgical indication, was overrepresented among referral patients. While proper identification of patients necessitating higher-level care is important, these data suggest that factors other than preoperative medical comorbidities may influence this decision, potentially resulting in a continued financial burden for tertiary referral center.
**Background:** Autoantibodies (autoAbs) against desmoglein-1 (DSG1) and desmoglein-3 (DSG3) have conventionally been studied and well accepted in the pathogenesis of pemphigus vulgaris (PV) and foliaceus (PF). In some patients, however, disease activity does not always correlate with anti-DSG IgG levels. Recent studies have suggested that non-DSG autoantibodies (autoAbs) may contribute to the pathogenesis of pemphigus, including autoAb directed at acetylcholine receptors (AChR) and thyroid peroxidase (TPO). The purpose of this study is to retrospectively analyze PV and PF patient sera to better understand the relationship between anti-AChR and -TPO Abs to disease activity and DSG reactivity between patients treated with prednisone and steroid sparing agents (SSA; n=22) or prednisone and rituximab (n=21).

**Methods:** Patients were evaluated at 2 time points, T1 and T2, for disease activity using the Pemphigus Disease Area Index (PDAI), and sera were tested for the presence of TPO, DSG1, DSG3, muscarinic (M3) and nicotinic (n) AChR IgG autoAbs as well as antibodies against Varicella Zoster Virus (VZV) by ELISA.

**Results:** Disease activity, as measured by PDAI, significantly decreased in patients from T1 to T2 (p<.0001). After Bonferroni correction, a significant difference was seen in paired IgG anti-DSG1 (p<.0001) and anti-DSG3 (p<.0001) levels when T1 was compared to T2 in both treatment groups. A significant difference between pemphigus patients and normal subjects was seen only with nAChR (p<.0001) at T1. No significant difference was seen between T1 and T2 values in patients with pemphigus for TPO (p=.7627), m3AChR (p=.3846) or nAChR (p=.3280). No correlation was found between anti-DSG1 or -DSG3 IgG and anti-AChR or -TPO Ab levels in either treatment group at T1 or T2.

**Conclusions:** These findings demonstrate that although IgG anti -AChR and -TPO autoAbs were present in PV and PF subjects, they were not increased in our patient population versus controls and did not decrease with treatment. This suggests that these other antibodies may not play a direct role in the pathogenesis of disease.
**Depth-Based, Motion-Stabilized Colorization of Microscope-Integrated Optical Coherence Tomography Volumes for Microscope-Independent Microsurgery**

Isaac D. Bleicher, Moseph Jackson-Atogi, Christian Viehland, Hesham Gabr, Joseph A. Izatt, Cynthia A. Toth

**NIH MIOCT R01 Grant, Duke Eye Center P30 Core Grant, Retina Research Foundation / Joseph M. and Eula C. Lawrence Travel Grant**

**Background:** Optical coherence tomography uses reflected light to provide cross-sectional, histology-like images of ocular structures and microscope-integrated OCT (MIOCT) scanners allow images to be captured and displayed to the surgeon intraoperatively. Our research group has developed a real-time 3D rendering process to show volumes that intuitively demonstrate relationships between anatomy, pathology and instrumentation, but these volumes can be difficult to quickly resolve when surgical fields are complex. We hypothesized that motion-stabilized colorization of these volumes would improve microsurgical performance and ability to interpret surgical volumes.

**Methods:** Color was applied in real-time as gradients indicating axial position and stabilized based on calculated center of mass. A test on pre-recorded intraoperative MIOCT volumes was performed, in which surgeons (N=7) were asked to identify retinal membranes, the presence of an instrument, its contact with tissue, and associated deformation of the retina comparing grayscale and color visualizations. A controlled trial was performed, in which surgeons (N=15) performed microsurgical skills without optical visualization using grayscale and color MIOCT visualizations. Skills included thickness identification – ranking objects based on thickness, instrument placement – placing an instrument close to a flat surface without touching, and object manipulation – grasping an object with forceps. Each skill was assessed on time, confidence, and performance (number correct, distance above surface and inadvertent touches, and number of grasps and inadvertent touches, respectively).

**Results:** The colorization and stabilization algorithm was optimized to require 2.69ms per volume and expert review confirmed the accuracy of motion stabilization. In intraoperative volume testing, colorization improved ability to differentiate membrane from retina (p<0.01), correctly identify instrument contact with membrane (p=0.03) and retinal deformation (p=0.01). In controlled skills testing, subjects working with colorized volumes were faster (-18.9s and -13.3s for recessed and elevated objects respectively, both p<0.01) and more correct (+24% and +16% correct answers for recessed and elevated objects, respectively, both p<0.01) in assessments of thickness, were less likely to inadvertently contact a surface when approaching with an instrument (-1 contact, p<0.01), and uniformly more confident (+2, +1, and +1 change on a 5-point scale for thickness identification, instrument placement and object manipulation, respectively, all p<0.01).

**Conclusions:** Colorization enabled effective membrane identification, instrument tracking, and tissue deformation. In microsurgical skill testing, it improved surgeon efficiency, precision and confidence and increased the feasibility of microscope-independent surgery. This novel technology improved the utility and ease of use of intraoperative MIOCT such that microsurgical skills could be performed without a microscope by inexperienced users.
Predictive Efficacy of Longitudinal Brain Volumetry in Patients with Mild Cognitive Impairment using a FDA Cleared Automated Quantification Software

Christopher Calixte MHSc, Ziwei Zhang MD, PhD, P. Murali Doraiswamy MD, Benjamin Andrew MHSc, Sheng Luo PhD, Jeffrey R. Petrella MD

Supported by the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number TL1TR001116

Background: Alzheimer’s disease (AD) is a neurodegenerative process that is characterized clinically as a progressive dementia syndrome. Our aim was to assess the prognostic efficacy of using NeuroreaderTM (NR), a FDA cleared automated brain segmentation software, longitudinally on the MRIs of patients diagnosed with mild cognitive impairment (MCI). Our outcome was conversion to AD at 3-year follow up.

Methods: Patient clinical information was obtained through the Alzheimer’s Disease Neuroimaging Initiative (ADNI), a publically accessible North American multicenter trial and database. Patients were imaged using 1.5T or 3.0T T1-weighted 3D gradient echo magnetic resonance (MR). Baseline and 1-year changes in 12 regional brain MRI volumes were analyzed using Neuroreader. Receiver Operating Characteristic (ROC) curves were generated using 1-year Mini Mental Status Exam (MMSE) scores with and without NR metrics. The Area Under the Curve (AUC) for each model’s ROC was compared using the DeLong method to assess the prognostic efficacy of NR.

Results: At baseline, 349 patients (mean age 74, 39% female) diagnosed with MCI with 3-year follow up available were included for analysis. 155 patients (44%) progressed to AD at 3-year follow up. Demographic information included age, gender, education level, and apolipoprotein E4 (APOE4) genetic status. At baseline, using NR alone in detection significantly outperformed MMSE scores alone (AUC 77.2% [95% CI = 72.4 – 82.0] vs AUC 68.7% [95% CI = 63.1 – 74.3], p <.05). The model including 1-year changes in NR metrics and MMSE scores significantly outperformed the model with changes in MMSE scores alone; AUC 86.4% [95% CI = 82.7 – 90.2] vs AUC 79.9% [95% CI = 75.2 – 84.6], p <.05. Regionally, 1-year lateral ventricular expansion was significantly associated with an increased risk of conversion to AD. Each 1ml expansion in lateral ventricular volume was associated with an adjusted OR of 1.2 [95% CI = 1.08-1.36], p <.05. No other regional change was significantly associated with 3-year AD conversion.

Conclusions: The use of a commercially available FDA cleared automated quantification software on brain MRIs improved 3-year prediction of AD conversion in patients with MCI compared to MMSE scores at baseline and while tracking both metrics over a one-year interval. Annual lateral ventricular expansion was the strongest regional predictor of AD conversion. Tracking patient’s lateral ventricular dimensions on MRI with an automated quantification software can serve as a surrogate for overall cerebral atrophy in assessing the risk of further cognitive impairment. Future research should focus on the use of commercially available, FDA cleared quantification software to expedite the implementation of brain volumetry in clinical trials and practice.
The Effects of Single-Joint Lower Limb Osteoarthritis and Surgical Intervention on Walking Speed, Joint Power, Compensatory Strategies, and Disease Progression

James C. Campbell, Robin M. Queen, Daniel Schmitt

Background: Osteoarthritis (OA) of the hip, knee, or ankle is profoundly debilitating and leads to high medical and social costs. The reduction in mechanical power caused by OA in one joint will reduce walking speed and may require additional effort by other joints that can lead to joint overloading and increased muscular force in unaffected joints and limbs. Yet little is known about how other joints compensate for power loss in one joint, or how effective joint replacement surgery is at restoring joint mechanics. Here we test the hypotheses that single-joint OA will reduce power in the affected joint, that other joints will increase power to compensate, and that surgical intervention will rebalance power production across all joints.

Methods: Motion and force data were collected from controls and a novel cohort of subjects with single-joint lower limb OA using a 3-D motion capture system and force platforms. Subject velocity and power at the ankle, knee, and hip in both affected and unaffected limbs were calculated during the last third of stance phase prior to surgical intervention. Subjects receiving ankle or hip replacement were reanalyzed 1 year later. Limb asymmetry and associations between joint power were compared using ANOVA, with a threshold of p≤0.05.

Results: 15 controls, 63 hip OA, 20 knee OA, and 183 ankle OA subjects were examined prior to any intervention, with 183 ankle and 23 hip OA subjects analyzed after intervention. Mean speed and mean total power (TP) were 1.40m/s and 6.25W for controls, 1.06m/s and 5.25W for hip OA subjects, 0.95m/s and 4.02W for knee OA subjects, and 0.87m/s and 3.39W for ankle OA subjects. TP and speed were correlated (R²=0.60, p<0.0001). In ankle OA and hip OA, the unaffected limb contributes significantly more of TP than the unaffected limb (59% and 54%, respectively), but in knee OA and controls there is no asymmetry in power contribution. In control subjects, the ankles provided only about 50% of TP, whereas in knee and hip OA patients the ankles provided 78% and 75% of TP, respectively, showing that ankles are overloaded as a compensatory strategy in these patients. In contrast, patients with isolated ankle OA generate only 19% of TP from the affected joint and 42% from the unaffected ankle, relying more heavily on hip power (21%) than knee and hip OA subjects did (13% and 12%, respectively). Surgical intervention increases gait speed and TP (0.26m/s mean velocity increase and 1.15W mean TP increase for ankle replacement; 0.27 m/s and 1.55W mean increases for hip replacement), and decreases power asymmetry of limbs. The surgical joint significantly increases in its proportion of gait power (19% to 25% for surgical ankle, 4% to 8% for surgical hip), while reducing overloading of unaffected joints that had been compensating prior to surgery.

Conclusions: Single-joint lower limb OA leads to reductions in power production and gait speed, leading to asymmetric loading in other joints for compensation. This causes further fatigue and disease in previously unaffected joints, placing patients in a discouraging cycle of increasing pain and disability. Hip and ankle replacement are effective in restoring joint power, limb symmetry, and speed. These novel findings provide insight regarding which other joints will be most affected by single-joint OA and the effects of interventions on gait mechanics.
Impact of Diabetes Mellitus and Glycemic Control in Lung Transplant Donors on Recipient Survival

Valentine R. Esposito, Michael S. Mulvhill, MD, Matthew G. Hartwig, MD/MHS

Supported by the National Center For Advancing Translational Sciences of the National Institutes of Health under Award Number TL1TR001116

Background: Lung transplantation is the gold-standard therapy for patients with end-stage respiratory failure. To meet unmet clinical need, improved ability to ascertain the suitability of candidate donor allografts are needed. Presence of diabetes mellitus (DM) in donors of lung allografts is associated with worse recipient survivorship, but impact of donor HbA1c around the time of procurement on post lung transplant (LTx) survivorship has not yet been elucidated. The purpose of this study was to evaluate outcomes of recipients who received allografts from donors with elevated HbA1c using population-level data from the United Network for Organ Sharing (UNOS) database. We tested the hypothesis that elevated donor HbA1c had a detrimental impact on long-term post-LTx outcomes.

Methods: LTx cases in the UNOS database were merged with a UNOS-provided custom data set containing optionally-recorded HbA1c data for LTx donors from 2010 to 2015. Cases meeting inclusion criteria were divided into two groups: donors with peri-donation hyperglycemia (HbA1c ≥6.5) and euglycemic donors (HbA1c <6.5). Donor, recipient, and operative characteristics were compared between groups. Kaplan Meier analysis with the log rank test was used to determine differences in survival between the hyperglycemic donor and non-hyperglycemic donor groups. Cox proportional hazard models were used to determine the impact of donor perioperative hyperglycemia on overall survival when accounting for additional variables.

Results: 4078 LTx recipients were included in the analysis, with 244 (5.9%) receiving allografts from hyperglycemic donors. Donors with hyperglycemia were older and had higher BMIs compared to euglycemic donors. There was no statistically significant difference in recipient overall survival between the hyperglycemic donor and euglycemic donor groups (p=0.925). The Cox model did not suggest donor hyperglycemia to be a significant risk factor for recipient mortality (HR: 1.01, p=0.924). Furthermore, when examined across multiple subgroups, HbA1c was not found to impact survival. There were no statistically significant differences in rates of post-operative complications between donor groups.

Conclusions: Donor perioperative hyperglycemia (HbA1c ≥ 6.5%) does not negatively impact LTx recipient survivorship. Improvements in DM management may be responsible for this effect. Implementation of the Lung Allocation Score (LAS) system in 2005 may help explain the relationship between donor DM and recipient survivorship.
Assessing the Impact of Pre-Natal Mercury Exposure on Maternal-Child Health in Madre de Dios, Peru

Emma Fixsen, Andres Mallipudi, Ernesto Ortiz, Caren Weinhouse, William Pan Doris

*Duke International Clinical Research Fellowship*

**Background:** The Madre de Dios (MDD) region of the Peruvian Amazon is in the midst of a mercury contamination crisis caused by explosive growth of unregulated, artisanal gold mining. Miners use elemental mercury (Hg) to extract gold from river sediments, releasing an estimated 30-40 tons of Hg into local waterways every year. Elemental Hg is then converted to bioavailable methylmercury by bacteria, and humans are exposed via consumption of contaminated fish. Previous studies in MDD have shown both fish tissue Hg levels and hair Hg levels in women of childbearing age in excess of international guidelines. Hg exposure is known to cause multiple adverse health effects, including nervous system damage in adults and impaired neurological development in children. Hg easily crosses the placenta, and previous studies have found fetal levels to be higher than maternal levels. To examine the effects of Hg exposure during pregnancy on maternal-child health outcomes, we are conducting a birth cohort study, the first of its kind in the MDD region of Peru. Our objectives are to implement and manage enrollment and sample collection for a birth cohort study in a low-resource, socio-politically unstable area of Peru and to assess the relationship between maternal and fetal Hg levels, maternal diet and maternal/fetal Hg levels, and maternal hemoglobin and maternal/fetal Hg levels.

**Methods:** This is a prospective cohort of women beginning November 2016 and scheduled to end August 2018. Participants are healthy, non-smoking women between 18-35 years of age enrolled prior to 30 weeks gestation. The study consists of an initial encounter during a prenatal visit and a second encounter at the time of delivery. At each encounter, samples are collected (hair, umbilical cord blood, venous blood, placenta, nails) and dietary and Hg exposure surveys are completed by participating women.

**Preliminary Results:** Since November 2016, 211 women have been enrolled in the study. As of April 2018, 114 women have completed the delivery phase of the study, while 45 women have been lost to follow-up at the time of delivery (loss to follow-up rate 28.3%). Average venous blood Hg in the first 50 women was 6.28 ug/L at time of delivery (range 0.7-28.69, upper 25% 8.04), while average umbilical cord blood Hg was 9.98 ug/L (range 0.9-54.32, upper 25% 12.90). Of note, the EPA reference dose for cord blood Hg is 5.8 ug/L. Analysis of surveys and collected samples is ongoing.

**Conclusions:** A major challenge has been loss to follow-up at delivery for various reasons including homebirth, delivery at hospitals outside the study, and miscarriage. Logistical challenges include inconsistent power supply, lack of reliable communication with participants and health care workers, difficulty engaging healthcare workers with the project, unsafe transportation and the need to ship samples to the US for analysis. Overall, our preliminary results suggest that Hg contamination remains a pressing public health concern in the MDD region, and more investigation into how to mitigate this is required.
Osteoarthritis Biomarkers in Human Synovial Fluid of the Hip Joint


Funding from the Duke Department of Orthopaedic Surgery and the Piedmont Grant

**Background:** Molecular biologic markers show promise as objective measures of osteoarthritis (OA). This observational study examined twenty potential biomarkers in hip joint synovial fluid. Outcome measure was the variance in biomarker concentration between OA severity groups. Secondary outcome measures were the variance in biomarker concentrations across: operative Beck grades, ages, BMI, gender, ethnicity, race, and patient reported outcome (PRO) measures.

**Methods:** Included adult patients (n=79) having hip surgery for femoroacetabular impingement (n=43), acetabular dysplasia (n=11), or primary OA (n=25). All other secondary causes of OA were excluded. OA severity was graded according to the radiographic Tonnis Classification: none (n=23), mild (n=31), moderate (n=11), severe (n=14). All patient having a Tonnis grade of 0 also had pre-arthritic hip disease. Biomarker levels were then compared to Tonnis grades, Beck grades, and patient characteristics using the Kruskal-Wallis, Wilcoxon, Fisher Exact, and Spearman’s rank tests as appropriate. Also calculated principal component analysis (PCA) for biomarkers and kappa statistics for Tonnis grades. Exploratory factor analysis and regression analysis through multivariable models are ongoing. Two-year follow-up will correlate pre-operative biomarker levels to post-operative outcomes.

**Results:** Seventeen biomarkers showed significant differences (p-value <0.05) between both standard and dichotomous Tonnis grades: CTX-II, MMP-1, MMP-3, MMP-9, TIMP-1, IL-1b, IL-6, IL-8, TNFa, CRP, TSG-6, VEGF-A, VCAM-1, CD14, CD163, neutrophil elastase, and NTx. PCA shows that most biomarker variability occurs within the first four principal components. Five biomarkers showed significant differences (p-value <0.05) between dichotomous Beck grades: IL-6, VEGF-A, MMP-1, dCOMP, and sGAG. Age, BMI, and patient reported outcome measures showed significant differences (p-value <0.05) between Tonnis grades and weak to moderate correlations with the significant biomarkers. Inter-rater agreement across three Tonnis raters was excellent using standard (weighted kappa=0.82; 95%CI: 0.75-0.89) and dichotomous grades (kappa=0.80; 95%CI: 0.68-0.93).

**Conclusions:** Results support future investigation into 17 of 20 biomarkers, however this analysis does not differentiate which of the seventeen are more important in OA progression. PCA suggests that most of the biomarkers contribute to the variability between OA severity groups and justifies using only the first four principal components for multivariable regression. Researchers should consider the biomarkers that showed significant variance between Tonnis grades and were significantly associated or correlated with secondary variables: MMP-1, IL-6, VEGF-A, IL-8, CRP, and TIMP-1. Three of these biomarkers, MMP-1, IL-6, and VEGF-A, showed significant variance between both Tonnis and Beck grades, making them potential biomarkers in pre-arthritic hip disease. We recommend that future studies obtain sufficient funding and sample size for a predictive model and include serum, urine, and synovial fluid samples. The Tonnis Classification is reliable between raters.
A Propensity Matched Survival Analysis: Do Simultaneous Liver-Lung Transplant Recipients Need a Liver?

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Clinical Translational Science Award

Background: There is debate whether simultaneous lung-liver transplant (LLT) long-term outcomes warrant allocation of two organs to a single recipient. We hypothesized that LLT recipients would have improved post-transplant survival compared to matched single-organ lung recipients with an equivalent degree of liver dysfunction.

Methods: The OPTN/UNOS STAR file was queried for adult candidates for LLT and isolated lung transplantation from 2006-2016. Waitlist mortality and transplant odds were calculated for all candidates. Donor and recipient demographic characteristics were compiled and compared. LLT recipients were matched 1:2 with a nearest neighbor method to single-organ lung recipients. Kaplan-Meier methods with log-rank test compared long-term survival between groups. Univariate regression was used to calculate the association of LLT and mortality within 6 months of transplant. A proportional hazards model was used to calculate risk-adjusted mortality after 6 months post-transplantation.

Results: Thirty-eight LLT patients were matched to 75 single-organ lung recipients. After matching, no differences in baseline demographics or liver function were observed between cohorts. Length of stay was significantly longer in LLT recipients compared to isolated lung recipients (45.89 days vs. 22.44 days, p<0.001). There was no significant difference in survival probability between LLT and isolated lung transplant (1-year 89.5% vs. 86.7%, 5-year 67.0% vs. 64.6, p=0.20).

Conclusions: After matching for patient characteristics and level of liver dysfunction, survival in simultaneous LLT was comparable to isolated lung transplantation. While this population is unique, the clinical picture prompting liver transplant is not clear. National guidelines to better elucidate patient selection are needed.
**Background:** Neoadjuvant therapy (NAT) allows for early treatment of breast cancer, aiming to reduce tumor burden and allow patients to undergo breast-conserving surgery rather than mastectomy. However, response rates to NAT vary. While some patients are able to achieve a pathologic complete response (pCR), a significant portion of patients may not respond to NAT. These patients may incur toxicities and costs associated with NAT, but gain proportionally less benefit. This retrospective study evaluates the utility of using features extracted from pre-treatment MRI to predict tumor response to NAT.

**Methods:** Institutional review board approval was obtained for this retrospective study of 288 breast cancer patients at our institution who received NAT and had a pre-treatment breast MRI. A comprehensive set of 529 radiomic features was extracted from each patient’s pretreatment MRI. The patients were divided into equal groups to form a training set and an independent test set. Two multivariate machine learning models (logistic regression and a support vector machine) based on imaging features were trained to predict pCR in (a) all patients with NAT, (b) patients with neoadjuvant chemotherapy (NACT), and (c) triple negative or human epidermal growth factor receptor 2-positive (TN/HER2+) patients who had NAT. The multivariate models were tested using the independent test set, and the area under the receiver operating characteristics (ROC) curve (AUC) was calculated.

**Results:** Out of the 288 patients, 64 achieved pCR. The AUC values for predicting pCR in TN/HER+ patients who received NAT were significant (.707, 95%CI: 0.582–0.833, p < 0.002).

**Conclusions:** The multivariate models based on pre-treatment MRI features were able to predict pCR in TN/HER2+ patients.
Lipid Trajectory and Predictors of Correct Management of Patients with High LDL

Matthew Gold, Tony Schibler, Daniel Wojdyla, Paul Hoffman, Ann Marie Navar

**Background:** Low Density Lipoprotein cholesterol (LDL-C) is an independent risk factor for atherosclerotic cardiovascular disease (ASCVD). Those with extremely high LDL-C are at particularly high risk. The 2013 ACC/AHA guidelines recommended that all adults with an LDL-C $\geq$ 190 mg/dL initiate high-intensity statin therapy with a goal of at least 50% LDL-C reduction. The prevalence of adults with extremely high LDL-C, and the degree to which these adults are identified for treatment, however, remains unknown.

**Methods:** We evaluated the prevalence of adults with an LDL-C $\geq$ 190 mg/dL in two populations: first, in adults seen across 213 health systems using the Cerner electronic medical record, and second, among adults followed at Duke. In each database, the number and characteristics of patients with LDL-C $\geq$ 190 mg/dL are described. Next, we assessed the proportion of adults with extremely high LDL-C who achieve control or LDL-C lowering in the Cerner database.

**Results:** Out of 172,920,586 adults with an LDL-C level drawn in the Cerner Health Facts database, 139,539 (0.08%) patients had at least one LDL-C $\geq$ 190 mg/dL. Many of those with extremely high LDL-C were young: the median age was 56 years, with 11% under age 40. Of those with extremely high LDL-C, 84.6% were Caucasian, 8.6% were African American, and 64.5% were female. Among those who had at least one additional LDL-C drawn at follow-up (n=49,002), fewer than half (49.5%) achieved a 30% LDL-C reduction, 19.8% achieved a 50% or greater reduction in LDL-C, and only 22% achieved an LDL-C <100 mg/dL. There was no difference in the odds of LDL-C control by sex (p=0.31), however increasing age and Caucasian race were associated with increased odds of achieving LDL-C control (p<0.001 for age, p=0.001 for race).

At the Duke population, we identified 7,874 patients with an LDL-C $\geq$ 190 mg/dL. Characteristics of those at Duke were similar to those seen in the HealthFacts Database: median age 57 years, 63.9% female. Only 14.7% of those with LDL-C $\geq$ 190 mg/dL were on any lipid lowering therapy (11.5% on a statin).

**Conclusions:** Large numbers of US adults have extremely high LDL-C, very few of whom are currently on lipid lowering therapy. The vast majority of patients newly identified with an LDL-C $\geq$ 190 mg/dL do not achieve guideline-recommended reductions in LDL-C. Ongoing efforts are underway to explore predictors of LDL-C control among patients at Duke to identify barriers to care and improve control rates in this high-risk population.
Inhibitory Plasticity of Basal Ganglia Output Nuclei in Parkinson’s Disease: Stimulation and Levodopa

Robert Gramer, BSc, Luka Milosevic, BEng, Musleh Algarni, MD, Alfonso Fasano, MD, PhD, Suneil K. Kalia, MD, PhD, Mojgan Hodaie, MD, PhD, Andres M. Lozano, MD, PhD, Milos R. Popovic, PhD, William D. Hutchison, PhD

Rauch Family Merit Scholarship & Clinical & Translational Science Institute Scholarship

Background: Deep brain stimulation of the subthalamic nucleus or globus pallidus internus is an efficacious treatment for the motor symptoms of Parkinson’s disease (PD). Recent studies have implicated the substantia nigra pars reticulata as a promising complementary target for treatment of axial motor symptoms. The underlying physiological mechanisms of electrical stimulation remain unclear, and a description of the direct effects of varying pulse width is lacking, despite clinical evidence of an effect on outcome.

Methods: Two closely spaced (600µm) microelectrodes were advanced into the substantia nigra pars reticulata and/or globus pallidus internus in each of 28 patients undergoing deep brain stimulation surgery for PD. Sets of 1Hz “test pulses” (100µA; 10 pulses per set) were delivered at different cathodal pulse widths in randomized order (25, 50, 100, 150, 250µs) before and after a “long-train” of high frequency stimulation (100Hz, 100µA, 150µs pulse width, 10s).

Results: We found that the stimulation pulse width progressively increased the amplitudes of extracellularly recorded focally evoked potentials and the durations of inhibitory silent periods. This was likely due to activation of the predominant striatal inhibitory afferent innervation to the basal ganglia output nuclei and hyperpolarization of postsynaptic neurons. Moreover, high frequency stimulation led to enhancement of inhibitory synaptic plasticity, which scaled with pulse width. In the substantia nigra pars reticulata, we demonstrated a direct phase-amplitude relationship between the duration of neuronal inhibition and the amplitude of potentials. We further showed that synaptic enhancement was greater in the globus pallidus internus compared to the substantia nigra pars reticulata, and that administration of one tablet of levodopa (Sinemet 100/25) had a potent effect on the enhancement of inhibitory plasticity in the substantia nigra pars reticulata. Finally, we demonstrated that lower levels of plasticity in the substantia nigra pars reticulata was associated with more severe axial and global motor symptoms.

Conclusions: The findings of this study demonstrate that the efficacy of inhibitory synaptic transmission may be involved in the pathophysiology of PD and furthermore may have implications for the development of novel stimulation protocols. Beyond understanding the pathophysiology of PD and augmenting traditional DBS methods, this work can contribute to the creation of closed-loop, physiologically-responsive DBS, and advancements that will enrich the treatment of numerous other movement disorders, epilepsies, dystonias, as well as the rapidly emerging indications for psychiatric ailments.
Creating a Novel Suture Anchor for Abdominal Wall Closure

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Background: Mesh suture is a novel form of suture that is used to close high-tension wounds such as in hernia repair or laparotomy closure. This is because they have greater tensile strength and increased resistance to suture pulling through tissue in comparison to standard suture. However, mesh sutures can produce large knots that are susceptible to increased palpability, infection, and foreign body response. The goal of this study was to develop and test a novel suture anchor clip to replace mesh suture knots in abdominal wall closure.

Methods: The anchor clip was iteratively developed using 3D design software (Fusion360®) and produced via 3D printing (Carbon3D® Printer). Anchor prototypes were initially tested in a suture fixation model using a silicone substrate. Monotonic tensile testing was performed to determine fixation strength and cyclic tensile testing performed to determine fixation durability. Results were compared to a standard of care knot and alternative suture fixation devices. The final anchor design (Fig. 1) was selected based on minimal size and superior mechanical performance. Next, the anchor clip underwent repeat cyclic fatigue testing in an abdominal wall closure model which approximated porcine abdominal wall using a simple interrupted or simple running suture pattern. Completed cycles and post-cyclic failure load were recorded and compared to a standard of care knot.

Results: The size of the anchor clip (160 mm³) was ~60% smaller than a mesh suture knot (420 mm³). In the suture fixation model, monotonic testing revealed a significantly greater anchor clip failure load compared to a suture knot and alternative suture fixations (p< 0.05). Additionally, all anchors clips successfully completed cyclic fatigue testing without failure while other fixations, including knot, failed to complete cyclic fatigue testing multiple times. In the abdominal wall closure model, the anchor clip and knot consistently completed cyclic tensile testing and recorded similar post-cyclic testing failure loads for both suture patterns.

Conclusions: The anchor clip demonstrates similar or superior ability to secure mesh suture in comparison to a standard of care knot in a suture fixation model and abdominal wall closure model. The anchor clip is also much smaller in size than a mesh suture knot. This study provides a preliminary indication for the use of anchor clips in abdominal wall closure, such as hernia repair and laparotomy closure.

Figure 1. Anchor Clip Design
Targeting Neuroinflammation with Human Umbilical Cord Tissue-Derived Mesenchymal Stromal Cells

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**Background:** Demyelination is common in many devastating CNS diseases, including progressive multiple sclerosis, cerebral palsy and Krabbe disease. While many of these diseases lack effective treatments, mesenchymal stromal cell (MSC) therapy has emerged as a viable approach to treating some demyelinating diseases. MSCs, which benefit many rodent models of demyelinating disease through their immunosuppressive effects, are abundant in umbilical cord tissue. Our hypothesis is that human umbilical cord tissue derived MSCs can limit neuroinflammation by suppressing the activation of T cells in the CNS. Through suppression of neuroinflammation, ongoing demyelination will be halted and reversed.

**Methods:** In order to test this hypothesis, we assessed the ability of MSCs to prevent clinical disease progression in experimental autoimmune encephalomyelitis (EAE), the mouse model of multiple sclerosis. To induce EAE, we immunized mice with the CNS antigen, MOG peptide, as well as complete Freund’s adjuvant and pertussis toxin. Within 9-12 days of EAE induction, mice began to exhibit clinical signs of disease, in the form of ascending paralysis. Based on the severity of these clinical signs, we scored mice according to a well-delineated scale of 0-5. This clinical score is a direct reflection of the degree of neuroinflammation. In one experiment, MSCs were injected prior to the onset of EAE disease as a preventative dose, while in a subsequent experiment, they were injected shortly after onset as a therapeutic dose. We continued to monitor their clinical scores after MSC injection.

**Results:** In both experiments, we found a statistically significant improvement in clinical score in mice that received injections of MSCs in comparison to mice that did not.

**Conclusions:** Our results demonstrate that MSCs have a prophylactic as well as therapeutic role in the prevention of EAE disease progression. Further experiments will aim to elucidate the mechanism of MSC-mediated T-cell suppression in EAE and other mouse models of CNS neuroinflammation, with the intention of eventually using these cells to treat pediatric and adult patients with selective demyelinating diseases.
The Use of an Upper Extremity Functional Survey to Measure Glaucoma Medication Administration Success

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Background: Glaucoma medication administration difficulties can hinder adherence in the predominantly elderly glaucoma population, and busy providers can often be left unaware of their patients’ ability to self-administer their drops. Given the upper extremity dexterity and coordination necessary to correctly instill eye drops, we hypothesized participants with more upper extremity limitation, as reported in a validated upper extremity functional survey, would have reduced observed success with individual components of drop administration and a composite measure of drop administration, and that patient upper extremity co-morbidities may mediate these relationships.

Methods: We recruited 61 veterans with medically treated glaucoma at the Durham VA Eye Clinic as part of a larger ongoing clinical trial. At the initial study visit, participants were scored on their observed ability to place a drop into the eye (accuracy), express no more than one drop from the bottle (efficiency), and prevent contamination of the bottle tip (safety). Successful execution of these three conditions was defined as good drop technique. Participants also completed the 11-item QuickDASH survey, a validated upper extremity functional survey, with higher scores indicating worse extremity disability. Upper extremity co-morbidities were abstracted from participants’ medical records and clustered into groups with similar etiologies.

Results: The sample had a mean age of 69.1 years and were 96.7% male, 60.7% African American, and 31.2% white. Participants took a mean of 2.2 glaucoma medications. When observed, 31.2% of participants displayed good drop technique. However, there was no significant difference in QuickDASH scores for those who were or were not successful with the individual drop administration components of accuracy (21.0 vs. 33.5, p=0.14), efficiency (20.2 vs. 26.1, p=0.31), safety (23.3 vs. 21.8, p=0.80), or the composite good drop technique (19.5 vs. 24.0, p=0.47). Further, there were no significant associations between upper extremity neurologic or musculoskeletal co-morbidities and the good drop technique composite, though the presence of clusters of upper extremity co-morbidities were associated with higher QuickDASH scores.

Conclusions: Though a limited sample size likely underpowered the analysis of the relationship between drop administration measures and QuickDASH scores, this study suggests observation of drop administration in the clinic is the best means to determine a patient’s ability to adhere to glaucoma medical therapy.
National Trends in Hospitalizations for Chronic Liver Disease from 2005 to 2014

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Background: It is unknown how changes in healthcare have impacted the care of patients with liver disease. This analysis evaluates characteristics and trends among patients hospitalized for complications of chronic liver disease (CLD) in the United States.

Methods: CLD-related hospitalizations in adults (≥18 years) from 2005 through 2014 were identified in the National Inpatient Sample (NIS) database. A CLD-related hospitalization was defined by a primary discharge diagnosis with one of the following ICD-9 codes: bleeding or non-bleeding esophageal varices (456.0, 456.1, 456.20, 456.21), spontaneous bacterial peritonitis (567.23), alcoholic cirrhosis of the liver (571.2), cirrhosis of the liver without mention of alcohol (571.5), biliary cirrhosis (571.6), hepatic encephalopathy (572.2), portal hypertension (572.3), hepatorenal syndrome (572.4), other sequelae of CLD (572.8), hepatopulmonary syndrome (573.5), or ascites (789.5, 789.59). Clinical and hospital characteristics were queried. Total hospitalizations by year, sex, race/ethnicity, payer, and etiology of liver disease were calculated. Population based rates of hospitalization were calculated using National Center for Health Statistics data and age-standardized using the US 2000 population.

Results: Between 2005 and 2014, 319,749 CLD-related hospitalizations were identified in the database. With recommended weighting for national estimates applied, these correlate to an estimated 1,546,902 CLD-related hospitalizations nationally (95% CI: 1,346,164-1,747,640). Despite decreases in LOS and in-hospital mortality, overall national discharge rates for CLD-related hospitalizations remained constant over the study period at 65 to 70 per 100,000. Mean age for CLD hospitalizations increased 0.17 years annually, from 56.93 years in 2005 to 58.30 years in 2014 (p<0.0001). The proportion of Medicare hospitalizations increased throughout the study. An increase in Medicaid hospitalizations was noted in 2014. Changes in etiology occurred. HCV hospitalizations decreased 26.8%, while NAFLD hospitalizations increased 168.3%. Rates of CLD-related hospitalizations varied by race/ethnicity and sex. Hispanic and white males had the highest hospitalization rates.

Conclusions: In this nationally representative sample, we showed continued decreases in LOS and in-hospital mortality in hospitalizations for CLD. Overall rates of CLD-related hospitalizations remained unchanged, but etiologies shifted with reductions in viral hepatitis and increases in NAFLD. Rates of CLD-related hospitalizations varied according to sex and race/ethnicity. This study points to important weaknesses in our current care of liver disease. These weaknesses manifest themselves in the form of at risk populations and trends towards high levels of ALD and NAFLD. Addressing these coming issues will be key in reducing the immense burden of inpatient liver disease care in the US.
**Bridging the Gap: An Analysis of Gene Expression in Matched Colorectal Cancer Primary and Metastatic Tumors**

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**Background:** Colorectal cancer (CRC) is the third most common cancer among men and women in the US. Although significant strides have been made in the detection and treatment of CRC, metastatic disease is still responsible for significant mortality and morbidity. We hypothesize that there are fundamental differences in the underlying tumor biology between the primary tumor and its subsequent metastases that may contribute to tumor metastasis and drug resistance. To address this question, we have assembled a cohort of matched primary and metastatic CRC tumors to evaluate the gene expression of potential therapeutic targets thought to play a role in metastasis and immunomodulation. Our aim was to determine how the molecular signatures of these CRC tumors change during the metastatic process.

**Methods:** Using the Duke electronic medical record system, we identified patients who had a metastatic resection sample available at Duke University and a primary tumor sample at a known medical center. Formalin-fixed, paraffin-embedded (FFPE) primary and metastatic tumor samples were evaluated by a trained GI pathologist and only high-quality samples were included in these analyses. RNA was extracted from patient-paired samples and quantitative real time PCR (qRT-PCR) was performed to evaluate relative gene expression in the primary and metastatic tumors. We measured the gene expression of CLDN2, CLDN18.2, CDH17, ITGB6, DPEP1 and TEM8, all potential therapeutic targets thought to play a role in metastatic formation. Finally, we measured relative gene expression of these same markers in patient derived CRC xenografts (PDX) generated from fresh tumor tissue collected from primary and metastatic sites.

**Results:** We evaluated 50 patient samples and 48 PDX tissue samples. Our patients had a median age of 61 years old, 33 (66%) males and 17 (34%) females and 30 (60%) colon samples and 20 (40%) rectal samples. We showed that gene expression levels were higher in the primary tumor for TEM8 (p<0.001), CDH17 (p= 0.019) and MST1R (p=0.006). Interestingly, CLDN2 (p=0.03) relative gene expression was higher in the metastatic tumor than the primary. No differences were observed between primary and metastatic tumors for DPEP1 (p=0.292) and ITGB6 (p=0.437). We were unable to accurately measure CLDN 18.2 levels in our samples as they were below the limits of detection. Lastly, we did not observe any differences in the expression of these genes in our PDX model, demonstrating the value of the paired samples we used for our analyses in patients.

**Conclusions:** Our analysis of paired primary and metastatic tumors provides novel information regarding the evolution of the molecular signatures of tumors once the metastatic process has been initiated. Understanding the molecular differences between the tumors in the primary and metastatic locations allows for targeted therapies that take into account critical changes in the tumor biology that may directly affect treatment efficacy. This preliminary analysis illuminates potential factors that drive metastatic growth and may one day inform and improve future therapeutic approaches for CRC patients.
Cyclophosphamide-Induced Cystitis Results in NLRP3-Mediated Inflammation and Disruption of the Blood Brain Barrier in the Hippocampus

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**Background:** Chronic pain syndromes, with the most common in urology being interstitial cystitis (IC), are thought to be secondary to neuroinflammation. One of the emerging pathways studied in central nervous system (CNS) inflammation is the NLRP3, IL-1β pathway. The NLRP3 inflammasome, often referred to as the central processing unit of inflammation, is a component of the innate immune system that responds to damage and injury by activating caspase-1 and releasing pro-inflammatory cytokines from damaged cells. Given recent advances showing that several diseases of peripheral tissues result in inflammation within the brain, we reasoned that an insult to the bladder may result in inflammation in the CNS. In this study, we used a model of cyclophosphamide (CP)-induced cystitis, a standard model of acute cystitis, to evaluate whether insults to the bladder may result in NLRP3-induced inflammation within two structures implicated in micturition, the hippocampus and pons.

**Methods:** Female Sprague Dawley Rats (~200 g) were injected with cyclophosphamide (CP) [150 mg/kg i.p.] or PBS (vehicle) 24 hours prior to isolation of hippocampus and pons. Caspase-1 activity within study tissues was measured using a fluorometric assay (excitation: 400 nm and emission: 505 nm). rtPCR was performed on neural tissue to evaluate changes in the expression inflammasome components and the pro-inflammatory cytokines, IL-1β and IL-18, produced during NLRP3 activation. Histologic analysis of inflammation within the brain was performed following hematoxylin and eosin staining. Analysis of blood brain barrier (BBB) permeability was performed using an Evan’s blue assay in which tissues were harvested, placed in formamide, incubated overnight at 56°C, and the absorbance (620 nm) of formamide was measured. In order to study the impact of pharmacologic inhibition of NLRP3, rats were treated with glyburide, a potent inhibitor of NLRP3, along with CP or saline. Histologic analysis and changes in BBB permeability were measured as previously described.

**Results:** Caspase-1 activity was found to be significantly increased in the hippocampus of rats injected with CP when compared to vehicle rats (p<0.05). Interestingly, there was no statistically significant increase in caspase-1 activity in the pons of CP treated rats. Looking further into inflammation within the hippocampus, there was an increase in IL-1β and IL-18 expression (p<0.05) with no change in inflammasome expression. Histologic analysis demonstrated microglial activation and inflammation within the hippocampus. Further, Evan’s blue dye extravasation demonstrated that there was a significant increase in blood brain barrier permeability in the hippocampus of CP-treated rats when compared to vehicle rats (p<0.05). Importantly, administration of GLY with CP returned blood brain barrier permeability in the hippocampus to levels equal to vehicle rats.

**Conclusions:** Cyclophosphamide-induced cystitis results in activation of the NLRP3 inflammasome and increased blood brain barrier permeability in the hippocampus. This result is the first to demonstrate that insults to the bladder can directly stimulate inflammation in higher order brain structures and suggests a connection between the urinary tract and the brain, not previously shown or foretold.
The Role of Matrix Metalloproteinase-1 (MMP-1) in Ovarian Cancer Recurrence

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Ovarian Cancer Research Fellowship

Background: Ovarian cancer (OC) is the most common cause of gynecological cancer death. One reason for the dismal OC survival rate is the high rate of cancer recurrence following initial treatment. We have previously demonstrated that recurrent OC has a higher expression of MMP-1 when compared with the primary OC from the same patients. MMP-1 contributes to tumor cell spread by helping to permeate through connective tissue. Cancer cells, in general, thrive in acidic conditions. The objectives of this study were to investigate MMP-1 expression in acidic environments and determine how the extracellular matrix (ECM) affects invasiveness and chemosensitivity.

Methods: OC cells and normal fibroblasts in the ECM were transduced with lentiviruses carrying MMP-1 or non-silencing shRNAs. Gene knockdown was confirmed using RT-PCR. OC cells were cultured in acidic, MMP-1 knockdown, or non-silencing control media for invasion analysis. Chemosensitivity was evaluated using OC cells cultured in conditioned MMP-1 knockdown or non-silencing control media and then treated with paclitaxel.

Results: OC cells cultured in acidic media have increased expression of MMP-1 and increased invasion. However, OC cells show increased invasion and decreased chemosensitivity when cultured in HEK293 media with low levels of MMP-1 from shRNA-mediated knockdown. Specifically, CaOV2 cultured in HEK293 MMP-1 knockdown media showed increased invasiveness (p<0.05) and decreased chemosensitivity to paclitaxel (p<0.05) when compared to cells cultured in media with control levels of MMP-1.

Conclusions: Contrary to literature suggesting that MMP-1 facilitates tumor invasion in other cancers, our results suggest that culturing OC cells in an environment devoid of MMP-1 increases cell invasion and decreases chemosensitivity. This data opens the door for exploration into the functional difference between MMP-1 expressed by cancer cells and MMP-1 expressed in the tumor microenvironment.
Induction of Neutralizing Antibodies Against Autologous Viruses in HIV-1 Infected Pregnant Women Immunized with a gp120/160 Vaccine

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Doris Duke Charitable Foundation Clinical Research Mentorship Award

Background: Each year, >150,000 infants acquire HIV-1 via mother-to-child transmission (MTCT) despite the wide availability of antiretroviral therapy (ART). Even with optimal maternal and infant combination ART, there is still up to a 1% transmission rate. Thus, additional preventive strategies beyond ART are likely required to completely eliminate MTCT of HIV. Autologous viruses in pregnant women are the source of vertically transmitted variants, and prior work suggests that a maternal vaccine that boosts non-broad, neutralizing antibody (Ab) responses against these viruses could effectively decrease MTCT risk. However, it is not yet known whether HIV-1 envelope (Env) vaccines could be used to enhance autologous virus neutralizing Ab responses during pregnancy. As a result, we investigated whether immunization of HIV-infected pregnant women with an Env vaccine in a historic trial improved the mother’s ability to neutralize her own circulating viruses.

Methods: In the AIDS Vaccine Evaluation Group Protocol 102/104, HIV-infected pregnant women were immunized with a gp120 (n=10) or gp160 Env vaccine (n=1), or placebo (n=6). Full-length env sequences were isolated from maternal plasma by single-genome amplification (SGA) at a pre-immunization timepoint and after several vaccine boosts for vaccinees (n=7) and placebos (n=3). Env genes were generated as Env-pseudoviruses and the neutralization activity of paired maternal plasma against autologous viruses across visit timepoints was assessed in TZM-bl cells. The magnitude of plasma Env-specific IgG binding responses to a panel of various HIV-1 antigens (gp120, gp41, and V3 loop and V1V2 region epitopes) was measured by a binding antibody multiplex assay.

Results: After the last vaccine boost, five of 11 vaccinees had a detectable increase in gp120, V1V2, and V3-specific IgG responses and in tier 1 heterologous virus neutralization activity. Maternal plasma of the gp160 vaccinee neutralized viruses isolated from the pre-immunization timepoint more potently than the plasma of one placebo-control. In addition, the post-immunization virus population of the gp160 vaccinee was relatively more neutralization-resistant compared to that of the placebo-control. Preliminary viral genetic diversity analysis demonstrated decreased maternal viral diversity after gp120/160 vaccination (mean difference in Hamming distance between post- and pre-vaccine sequences was 1.3e-05 per base per day for 3 vaccinees; 4.2e-05 per base per day for 2 placebos), a trend that will be statistically validated with env sequence data of additional patients.

Conclusions: Env vaccination may contribute to restricting viral diversity and increasing autologous-virus neutralizing activity in HIV-infected pregnant women. In particular, it may promote the development of resistant escape variants through Env vaccine-elicited maternal plasma autologous neutralization. This work will inform studies of next-generation maternal HIV-1 Env vaccines in pregnant women to work synergistically with ART to further reduce infant HIV infections and achieve an HIV-free generation.
Hip Functional Characteristics are Decreased in the Involved Limb of Patients Undergoing Hip Arthroscopy for Unilateral Femoroacetabular Impingement Syndrome

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Background: Femoroacetabular impingement syndrome (FAI) is a chronic hip condition that results from pathologic contact between the bony structures of the hip joint. The resulting damage and irritation causes the insidious and progressive onset of hip pain. Patients with FAI also often complain of mechanical symptoms such as clicking and popping, as well as giving way. While many of these patients respond to conservative management, a subset fail such management, requiring surgery. The purpose of this study is to determine the effect of symptomatic FAI on hip active range of motion (aROM), hip strength, balance, and quality of life in patients preparing to undergo hip arthroscopy for FAI.

Methods: Nineteen subjects with unilateral FAI and scheduled for hip arthroscopy participated in this study. Active range of motion (aROM) and strength were measured for hip flexion, extension, internal rotation, external rotation, abduction, and adduction. Strength data was corrected for weight, reported as percent body weight (%BW) and measured for hip flexion, extension, internal rotation, external rotation, abduction, and adduction. Single-legged balance (eyes open and eyes closed) was tested using a force plate. Subjects completed the PROMIS Pain Interference (PI), Physical Function (PF), and Emotional Distress (ED) questionnaires. Raw scores were converted into t-scores (mean: 50, SD: 10) and compared to data from the general US population. For aROM, strength and balance data, bilateral comparisons were made utilizing paired t-tests or Mann-Whitney U tests based on the normality of the data. One-sample t-tests were performed on PROMIS instrument data. Statistical significance was set at p<0.05 a priori.

Results: Subjects with FAI showed decreased hip flexion aROM (mean difference: 8.9°, p=0.007) and strength (mean difference: 3.3%BW, p=0.007), decreased hip extension aROM (mean difference: 4.6°, p<0.001) and strength (mean difference: 2.9%BW, p<0.001) and decreased hip adduction strength (mean difference: 1.3%BW, p=0.036) in the involved limb. All comparisons were calculated: [Uninvolved Side]-[Involved Side]. Survey data showed increased PROMIS PI scores (mean: 63.2, p<0.001) and decreased PROMIS PF scores (mean: 39.3, p<0.001) compared to the general US population. No other significant differences were found.

Conclusions: Subjects with unilateral FAI show decreased hip flexion and extension aROM, as well as decreased hip flexion, extension and abduction strength when comparing the involved side to the uninvolved side. This likely represents the subjective weakness and stiffness that patients often report when describing the symptoms of FAI. They do not show any side-to-side changes in static balance, but this may be the result of the bilateral coordination necessary for balance as opposed to a lack of balance deficits in the tested population. FAI patients show higher pain interference and lower physical function than average. These findings corroborate both patient reports and previous findings in the literature.
Macular Vasculature in Infants and Children
Assessed using Optical Coherence Tomography Angiography

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Background: Optical coherence tomography angiography (OCTA) has been useful to assess retinal vascular diseases including diabetic retinopathy and age-related macular degeneration. However, most OCTA studies have been performed on adults. OCTA in infants and children may aid understanding of pediatric vascular development and detection of disease. Herein, we assess the effect of various factors on pediatric microvasculature using OCTA.

Methods: In this prospective cross-sectional study, OCTA images of superficial and deep vascular complexes (SVC and DVC, respectively) were obtained on 135 healthy eyes of 89 infants and children (mean age 8.5 +/- 5.3 years, range 9 weeks-17 years) using Spectralis tabletop and Flex units (Heidelberg, Germany). The macular foveal avascular zone (FAZ) area and superficial and deep vessel length density (VLD) were determined with MATLAB on OCTA images. We assessed effects of age (Pearson correlation), sex (t-test), race (Wilcoxon rank sum), and axial length (AL; Pearson correlation) on FAZ and VLD in the SVC and DVC. Agreement (intraclass correlation) between left and right eye FAZ, SVC VLD, and DVC VLD was determined on bilaterally imaged patients.

Results: FAZ area significantly varied with race (p=0.018), but not with age, sex or AL (p>0.05). In SVC, VLD varied with age (quadratic, R²=0.54, p<0.001), race (p=0.037), and AL (R²=0.46, p<0.001), but not sex (p>0.05). In DVC, VLD varied with age (quadratic, R²=0.25, p<0.001) and AL (R²=0.46, p<0.001), but not sex or race (p>0.05). There was excellent symmetry between the right and left eyes for FAZ area (ICC=0.97), SVC VLD (ICC=1.00), and DVC VLD (ICC=1.00).

Conclusions: Quantitative studies of pediatric macular vasculature should take into account age, race, and AL. Because of excellent symmetry between eyes, in cases of unilateral disease, the unaffected eye may serve as a control for comparison.
Quantitative Analysis of Mitochondrial DNA Sequence Variation in Smokers

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NIEHS Medical Student Research Fellowship

Background: In the US alone, smoking accounts for more than 480,000 deaths per year and over $300 billion a year in healthcare costs and productivity losses. It is well known that smoking damages and mutates nuclear DNA, and multiple studies have shown that smoking causes mitochondrial dysfunction in cardiomyocytes and lung tissues. Because smoking also has known immunomodulatory effects, in this pilot study, we used ultra-deep sequencing to elucidate the effects of smoking on the mitochondrial DNA (mtDNA) sequence in whole blood and leukocytes.

Methods: Whole blood samples were isolated from 6 Black/African American male smokers and 6 non-smokers. CD4, CD8, CD14, CD15, CD19, and CD56 leukocyte aliquots were isolated from whole blood using antibody-coated magnetic beads. After DNA was isolated from these whole blood and leukocyte aliquots, mtDNA was amplified by long range PCR. Purified fragments were then tagged to create Nextera libraries and ultra-deep sequenced with the MiSeq (whole blood DNA) and NovaSeq (cellular DNA) systems. MtDNA sequences were aligned to the rCRS (revised Cambridge Reference Sequence NC_012920) and surveyed for sequence variants at three different levels of heteroplasmy: 1% (referred to simply as heteroplasmies), 60% (alternate alleles), and 95% (SNPs) variant call frequencies.

Results: Smokers had more whole blood SNPs than non-smokers (p = 0.033) and had higher variant frequencies (variants/kb) in electron transport chain genes, MT-ND2, MT-ND4L and MT-ND5 (complex I) (p = 0.018-0.041). While non-smoker whole blood sequence variation at all frequencies increased with age, variation in smokers was very loosely associated with age or pack-year smoking history but was more strongly inversely correlated to serum cotinine levels. In our cell type analyses, there were no significant differences between cell types between smokers and non-smokers. All smoker leukocytes displayed an inverse correlation between serum cotinine levels and mean total variant count, but for both smokers and non-smokers, there were cell-type specific differences in age-variant count correlations.

Conclusions: Smoking seems to preferentially affect complex I ETC genes. Acute tobacco smoke exposure seems to have a larger impact on mtDNA than cumulative smoking history. The effect of smoking may occur in hematopoietic stem and/or progenitor cells and propagate uniformly to all daughter cells. Platelets and other whole blood cells not included in this study may contribute significantly to non-smoker-smoker differences. Lastly, cell type-specific differences in age-variant count correlations could be due to differences in equilibria between aging mechanisms and mitotic segregation.
Predictors of Child Healthy Eating in Family Child Care Homes

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Background: The prevalence of childhood obesity is a critical public health concern. Rates of obesity among children in the United States are alarmingly high with 17% of all children (under 19) and even 9% of preschool children being obese. Poor food choice is one of the critical components linked to childhood obesity and early childhood is an important formative period for dietary habits. Family child care homes (FCCH) are a critical setting in which to address obesity in preschool children. Child and provider demographics and psychological factors may influence child healthy eating and it is important to elucidate possible relationships of predictors of child Healthy Eating Index (HEI).

Methods: We used baseline data from 166 FCCHs and 496 children enrolled in the Keys to Healthy Family Child Care Homes in North Carolina. Hypothesized predictors of child HEI included child sex, race, age, BMI, time in FCCH, provider race, age, education, BMI, psychological factors, and provider HEI. Hypothesized predictors of provider HEI included provider age, race, education, BMI, and self-efficacy. Bivariate and multivariate regression models were created to assess these relationships. Our analysis used SAS 9.4 (Cary, NC).

Results: The greatest predictors of child HEI were provider HEI and provider psychological factors. The greatest predictors of provider HEI were self-efficacy factors.

Conclusions: Our results suggest that child demographics are not as predictive of child healthy eating as provider predictors. In fact, our findings highlight the need to focus on FCCH providers in future research to address child healthy eating in the FCCH.
(In)Adequacy of Lymph Node Yield for Papillary Thyroid Cancer in the U.S.: An Analysis of 52,820 Patients

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*Pfizer Foundation Grant and the Duke Clinical Translational Science Institute Fellowship*

**Background:** The incidence of papillary thyroid cancer has sharply increased in recent decades, with surgical resection being the primary mode of treatment. Lymph nodes (LNs) represent the most common site of persistent or recurrent disease and their involvement is associated with disease-specific mortality. Recently, a set of stage-specific threshold values for the number of LNs to be evaluated during surgery was determined for ruling out residual occult nodal disease with 90% confidence. While adequate LN yield has thus been defined, there is much heterogeneity in practice in the number of LNs resected during papillary thyroid cancer surgery. This nationwide study assesses the prevalence of adequate LN yield and determines its association with patient demographic and clinicopathologic characteristics.

**Methods:** Adult patients with pT1b, pT2 or pT3 M0 papillary thyroid cancer ≥ 1 cm who underwent surgery with ≥ 1 LNs resected were identified from the National Cancer Database, 2004-2015. Adequate LN yield was defined as removing ≥ 6, 9 and 18 LNs for pT1b, pT2 and pT3 stages, respectively, based on threshold values from recently published literature. Univariable and multivariable logistic regression models were employed to determine factors associated with adequate LN yield.

**Results:** 52,820 patients were included: 15,751 (29.8%) had adequate LN yield and 37,069 (70.2%) had inadequate LN yield. Rate of adequate LN yield increased from 18.2% to 32.4% over the decade. On unadjusted analysis, the proportion of patients with inadequate LN yield who were Black was greater than that of patients with adequate LN yield who were Black (5.3% vs. 4.0%, respectively, p<0.001). After adjustment, Whites were more likely than Blacks to have adequate LN yield [OR 1.24 (1.11-1.38), p<0.001]. Patients at academic centers were more likely to have adequate LN yield than those at community facilities [OR 1.60 (1.36-1.88), p<0.001]. Patients with pT3 tumors were least likely to have adequate LN yield (39.1% pT1b vs. 27.1% pT2 vs. 24.1% pT3, p<0.001). Of patients with adequate LN yield, 72.1% were found to have metastatic LNs, while 34.8% of those with inadequate LN yield had involved LNs.

**Conclusions:** The rate of adequate LN yield has increased over time, but only a minority of LN yields can be defined as adequate. Disparities still exist based on patient, facility and disease characteristics. Adequate LN yield is associated with a higher likelihood of detecting nodal disease, but unfortunately, patients with advanced tumors are least likely to have LN yield that is adequate.
Apoptotic Caspase 3 Promotes Surviving Tumor Cell Growth after Radiotherapy

Rayan N Kaakati, BS, Ruya Zhao, BS, Andrew K Lee, BS, Fang Li, PhD, Xinjian Liu, PhD, Chuan-Yuan Li, PhD.

Background: Apoptosis is a process of multicellular organisms, where cells are programmed to die in an orderly fashion. It has been recognized as an anti-oncogenic mechanism. However, an increasing number of studies are showing that the role of apoptosis in tumor formation is more complicated. We found that caspase 3, an “executioner” enzyme that plays key roles in apoptosis, can actually promote tumor cell repopulation during radiotherapy by stimulating paracrine growth signals from dying tumor cells. Based on our previous studies, we explored the potential to enhance melanoma radiation therapy by inhibiting Caspase3&7 by use of RNAi in the B16F10 melanoma model. We also explored the delivery of small hairpin RNAs (shRNAs) in B16F10 melanoma cells through the use of “Exosomes;” these are extracellular RNAs that have the ability to transport multiple RNA to different cells, which gives them tremendous promise in the delivery of drugs and microRNAs to tumors.

Methods: To generate exosomes with shRNAs, we established B16F10 cells that had been infected with lentiviral vectors encoding shRNA minigenes against Caspase3&7. Previous work in our lab has shown that downregulating Casp3/7 could significantly enhance radiotherapy of cancer. After verification of Casp3 &7 knockdown (KD) in the B16F10 cells by use of western blot, we extracted exosomes from the B16F10-shCasp3 or Casp7 cells. These exosomes were then incubated with un-transduced B10F10 cells. To determine if the shCasp3/7 containing exosome were effective in vivo, we injected the exosomes into B16F10 tumors grown in syngeneic C57/BL6 mice and also directly injected melanoma tumors with Casp3&7 knockdowns without exosomes for comparison and irradiated at 8 Gy. We also performed a clonogenic survival assay comparing the radiosensitivity of B16F10 wild type cells to B16F10 Casp3 and Casp7 knockdowns. Lastly, we measured PGE2 in the supernatant of B16F10 cells and Casp3&7 KDs with and without irradiation to investigate the mechanism behind the increased radiosensitivity in the caspase knockdown tumors.

Results: We observed a significant decrease in tumor growth after 8 Gy of irradiation in the shCasp3 injected group when compared to the shRNA Scramble group, but only saw a slight difference in tumor growth when we delivered our shRNA using exosomes. Our clonogenic assay showed a reduction in survival fraction in both shCasp3&7 due to increased sensitivity to radiation at high doses; however, when exosomes were used we did not see a difference. In elucidating the mechanism, we found significantly increased levels of PGE2 in irradiated wild-type cells in comparison with the non-irradiated control and irradiated B16F10 shCasp3&7 cells

Conclusion: Exosomes hold tremendous promise in the delivery of drugs to cancer cells, but still need to be further developed to effectively work in vitro and in vivo. In the present study, we were able to provide supporting evidence that Casp3/7 knockdowns decreased PGE2 induction by radiotherapy similar to previous studies.
Infant Weight and Duration of Procedure Associated With Increased Risk of Femoral Arterial Thrombosis after Cardiac Catheterization


**Background:** Although femoral arterial thrombosis is an uncommon complication of cardiac catheterization, it can cause limb threatening sequelae. Previous studies associating thrombosis with younger age are small scale or include only small cohorts of infants within older populations. We examined factors associated with thrombosis and the effect of post-procedure ultrasound (US) on detection rates after implementing post-procedure US.

**Methods:** We reviewed institutional records of patients 0-12 months undergoing catheterization from 2007-2016. Demographics and procedural data were compared between the thrombosis and non-thrombosis group. Pre and post-US groups were compared for thrombosis rate. We utilized a generalized estimating equations (GEE) logistic regression model to examine the association of pre and intraoperative patient characteristics to the presence of arterial thrombosis.

**Results:** In total, 270 patients underwent 509 catheterizations, resulting in 40 (7.9%) thromboses. Univariate analysis showed younger age, smaller body size, non-receipt of antiplatelet medication, longer procedure and fluoroscopy time, and larger diameter arterial catheter sheaths demonstrated greater odds of developing thrombosis (p<0.05). Multivariable analysis showed lower weight (odds ratio [OR] 0.463 per kg, 95% CI 0.328 – 0.655, p < 0.001), larger final arterial catheter sheath size (OR 2.88 per French size, 95% CI 1.581– 5.244, p < 0.001), and longer procedure duration (OR 1.009 per minute, 95% CI 1.003-1.016, p = 0.003) were independently associated with higher odds of thrombosis. Detected thrombosis rate after implementation of US increased from 6.5% to 15.8% (p=0.010).

**Conclusions:** Arterial thrombosis development after cardiac catheterization is associated with smaller (younger) infants, larger catheter sheath diameter, and longer procedures. Higher thrombosis rates were observed since implementing US. Further studies are needed to evaluate age-related changes in hemostasis in this population and how advanced screening methods and anticoagulation protocols may help improve short-term and long-term sequelae of femoral arterial thrombosis.
Impact of Procedural Bleeding in Peripheral Artery Disease

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Background: The relationship between invasive vascular procedures and bleeding in patients with peripheral artery disease (PAD) has not been well described in the literature. This post-hoc analysis from the EUCLID trial aimed to describe the incidence of major and minor post-procedural bleeding and characterize the timing and severity of bleeding events relative to the procedure.

Methods: EUCLID was a multicenter, randomized controlled trial of 13,885 symptomatic patients with PAD that tested the efficacy and safety of ticagrelor when compared with clopidogrel for the prevention of major adverse cardiovascular events. There were 2,661 patients that underwent coronary revascularization, peripheral revascularization, and/or amputations during the study period. The primary safety endpoint was TIMI major or minor bleeding, and all bleeding events underwent formal adjudication by a clinical endpoint classification group.

Results: Major bleeding events most often occurred within 7 days of the procedure. The incidence of bleeding <=7 days following peripheral revascularization (3.3%, CI 2.5-4.1%) was similar to coronary revascularization (4.0%, CI 2.6-5.4%) and lower extremity amputation (2.3%, CI 0.8-3.8%). The severity of bleeding events (as graded by drop in hemoglobin, need for transfusion, bleeding in a critical location, and fatal bleeding) was also similar following peripheral revascularization, coronary revascularization, and lower extremity amputation.

Conclusion: The incidence of major/minor bleeding following peripheral revascularization is comparable to coronary revascularization and lower extremity amputation, and the majority of bleeding events occur within 7 days following the procedure. The severity of peri-procedural bleeding events is also similar after procedures, with the most frequently adjudicated reason being a drop in hemoglobin >=2 grams/deciliter. Future studies should be performed to enhance our understanding of bleeding risk related to peripheral revascularization and lower extremity amputation in PAD patients.
ABC Rule in Longitudinal Melanonychia: Retrospective Single-Center Study Evaluating Clinical and Dermoscopic Features and Risk of Malignancy

Dayoung Ko BS, Clara Oromendia MS, Richard Scher MD, and Shari Lipner MD, PhD

**Background:** Longitudinal melanonychia (LM) is a common finding in clinical practice, but presents a difficult diagnostic challenge given its broad differential diagnosis. Subungual melanoma (SUM) is the most important condition affecting the nail that presents as LM. History, as well as, clinical and dermoscopic guidelines are helpful in distinguishing between benign and malignant etiologies of LM, but a nail matrix biopsy must be performed to make a definitive diagnosis.

**Methods:** A retrospective analysis was performed on a total of 84 consecutive cases of LM patients who received biopsies over a period of 7 years (8 subungual melanoma and 76 benign LM). Clinical and dermoscopic features as well as the ABC evaluation were compared amongst the two groups.

**Results:** When compared to benign cases of LM, SUM patients were younger with an average age of 36.1 years vs. 52 years in benign group (p=0.011). The SUM patients had their LM band for a longer duration with an average of 128.9 months compared to the benign group with an average of 38.3 months (p=0.017). The SUM group also presented with a wider band width with an average width of 5.3 mm compared to 3.1 mm in the benign group (p=0.002) as well as a greater width percentage, 49.5% compared to 24.8% in the benign group (p<0.001). However, no difference was found in whether the band underwent change, the digit affected, presence of Hutchinson’s sign, family or personal history of melanoma, and dermoscopic features between the groups. Furthermore, in the ABC evaluation, no difference was found in the number of ABC criteria met between the two groups.

**Conclusion:** This study supports that SUM usually presents with a wider band width and occupies a larger proportion of the nail plate than in benign cases of biopsied patients. This satisfies the “B” letter in the ABC rule, stating that band > 3 mm. However, the other letters in the criteria were not found to differ between the SUM and benign LM groups amongst biopsied patients and the number of ABC criteria met was also not different, which we would expect it to be if the criteria were accurate. Thus, we propose that the ABC rule for the clinical detection of subungual melanoma proposed by Levit et al, in 2000 does not fully discriminate between those with SUM and benign LM and that the absolute diagnosis of SUM is made by histopathologic evaluation. If there is any doubt in the clinical or dermoscopic evaluation of LM, a nail matrix biopsy should be performed.
Enhancing the Diagnostic Reference Level: Applying Automated Quality Metrics to Assess Liver Lesion Detection in Pediatric CT

Tyler Lacy, Aiping Ding, Ehsan Abadi, Yakun Zhang, Francesco Ria, Ehsan Samei, Don Frush

Background: Computed tomography scans have become a cornerstone of medical decision-making worldwide. Due to their widespread use, CT scanners now account for approximately one-fourth of total per capita radiation exposure to the US population. Current practices in radiology develop protocols to ensure that patient doses are as low as reasonably achievable. There are also requirements for dose monitoring at practices and institutions to assure consistence in dose delivery, and that doses are resonant with established levels. One such benchmark is the quantitative metric known as diagnostic reference levels (DRLs). These are established national or regional dose benchmarks based on the distribution of study doses for a given region. One measure used to report the doses is the computed tomography dose index (CTDI). Essential in establishing standards for CT performance is the recognition that DRLs, although implying “diagnostic” quality, are based only on dose. There is no measure of image quality. Therefore, optimization between the balance of patient radiation exposure and image quality cannot be achieved with DRLs alone. To this end, our purpose was to develop an automated program for evaluating pediatric body CT performance utilizing metrics of dose and image quality for the task of liver lesion detection.

Methods: The IRB approved investigation consisted of 748 clinically-performed IV-contrast-enhanced abdominopelvic CT scans of pediatric patients up to 18 years between June 2014 and November 2017 in three scanner models from two manufacturers. The clinical task was identifying a 5 mm liver lesion with a 50 Hounsfield unit attenuation difference from the background liver. Informatics systems extracted protocol information, patient diameter, age, dose, spatial resolution, and background noise from patient images. Quantitative image quality metrics were used to calculate a single detectability index (d’) which has been previously correlated with observer performance and represents the relative likelihood of liver lesion detection. The relationships between d’ and radiation dose were subsequently explored.

Results: There was minimal CTDI\textsubscript{vol} variability across ages. Abdominopelvic studies at 100 kV on one scanner model had a median CTDI\textsubscript{vol} of 3.0 mGy (2.8-3.4 mGy interquartile range). However, when applying d’, the age groups separated such that the younger patients had higher d’ values than the older patients. For the youngest age group, d’ and CTDI\textsubscript{vol} (medians) were 80 and 2.7 mGy; middle groups, 59 and 2.9 mGy; and oldest group, 42 and 3.4 mGy.

Conclusions: An automated method to assess clinical image quality quantified expected trends among different age groups for pediatric CT. This method provides tools to establish a quality reference level that would allow for enhanced assessment of CT performance. We believe that such a quality reference level would be a composite score which would encompass radiation dose and image quality and could serve to optimize CT programs on a large scale. Furthermore, these tools informed protocol changes at Duke which resulted in reduced inter-scanner quality variability and lower kV at greater ages than was previously used. These changes will lower radiation exposure to the effected ages without sacrificing significant image quality.
Abnormal Skin Perfusion in Patients with Graft-versus-Host Disease, Systemic Sclerosis, and Pan-sclerotic Morphea Compared to Healthy Controls

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National Institute of Allergy and Infectious Diseases,
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Background: Microvascular damage and dysfunction represent the earliest morphological marker of systemic sclerosis (SSc). The underlying pathophysiology of chronic cutaneous graft-versus-host disease (cGHVD), which mimics a variety of cutaneous auto-immune diseases such as SSc and pan-sclerotic morphea (PM), however, is unknown. Studies have suggested that host endothelial cells are a target of alloreactive donor cytotoxic T lymphocytes and substantial blood vessel loss may lead to impaired blood perfusion and tissue fibrosis in cGVHD. Identifying a non-invasive imaging device that is capable of early diagnosis of sclerotic cutaneous disease prior to irreversible damage will not only improve patient outcomes across these diseases, but also inform treatment plan. We sought to investigate skin perfusion using laser doppler flowmetry (LDF), a non-invasive imaging device, in patients with GVHD, SSc, PM, and localized morphea (LM) compared to healthy controls.

Methods: A prospective cohort of 29 patients (SSc (N=8) GVHD (N=5) LM (N=8) PM (N=4), PT (N=4) and 5 healthy controls were studied. Blood perfusion from both left and right wrists were measured at baseline of ~30°C and after application of heat to the skin of 44°C. Blood flow was expressed in arbitrary perfusion units (pU). Minimum, maximum, and mean pU were calculated at baseline and at time of maximum heat for each patient. Mann-Whitney t-test was used to compare pU at baseline and at 44°C.

Results: At baseline, there was no significant difference in mean pU among patients compared to healthy controls. At 44°C, however, patients with GVHD, SSc, and PM, but not LM (p=0.69) had a significantly larger increase in pU compared to healthy controls (Control: 12.3; GVHD: 95.6, p=0.0016; SSc: 68.6, p=0.00056; PM: 37.8, p<0.05; LM: 29.6, p=0.69). GVHD, SSc, and PM also had a wider range in pU between individuals, than healthy controls (Control: 9.5-36.4; GVHD: 31-181; SSc: 27.2 -142; PM: 11-55). Such differences were observed when data was compared using min, max values and left and right hands separately. All post-transplant GVHD patients, despite having no history of Raynauds and absent or stable sclerosis, developed an abnormal perfusion in response to heat, greater than that of patients with SSc (p=0.19) and PM (p=0.029).

Conclusions: Our study demonstrates dysregulation in non-digital cutaneous perfusion among patients with cGVHD, SSc, and PM but not LM. Underlying vascular changes may exist in patients with cGVHD perhaps early in the disease course, before chronic changes are present. We demonstrate that Laser Doppler Flowmetry can differentiate healthy controls from patients with cGVHD, PT, SSc, and PM. A non-invasive imaging device that is capable of early diagnosis of microvasculature dysfunction prior to clinical manifestation of microvasculature disease may have greater clinical applications such as earlier diagnose of sclerotic cutaneous disease prior to irreversible fibrosis and may provide a method of monitoring disease severity at point-of-care and allow for more informed treatment modifications.
Ultrasensitive detection of circulating tumor DNA for early detection, noninvasive genotyping, and disease monitoring in Non-Hodgkin Lymphoma

Joanne Soo, David M Kurtz, Joseph G Schroers-Martin, Michael C Jin, Florian Scherer, Alexander Craig, Matt van de Rijn, Maximilian Diehn, Ash A Alizadeh

Doris Duke Clinical Research Fellowship

Background: Non-Hodgkin Lymphoma (NHL) is the 6th most common type of cancer and 9th most common cause of cancer-related death in the United States. Clinical courses for NHL patients remain heterogeneous, with no existing methods for effective screening or prediction of treatment outcome. Circulating tumor DNA (ctDNA) has emerged as a promising biomarker for noninvasive disease detection and tumor genotyping. Detection of ctDNA in patients with NHL would allow the development of early screening tools for high risk populations, biopsy-free genotyping and clinical risk stratification, and accurate monitoring for minimal residual disease and relapse.

Methods: We applied Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq) to prediagnostic, diagnostic, and post diagnostic cell free DNA (cfDNA) samples from 287 patients with diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), or DLBCL-like post-transplant lymphoproliferative disorder (DLBCL-PTLD). Matched germline was sequenced for all patients, and matched diagnostic tumor samples were sequenced when available. We then assessed the utility of ctDNA for ultrasensitive detection of ctDNA in genotyping, screening, and monitoring contexts.

Results: Pretreatment ctDNA was detected in 98% subjects with over 80% concordance of truncal mutations when compared with tumor genotyping. In addition, plasma-based methods achieved over 80% concordance with tumor-based methods in classification of DLBCL and FL by cell-of-origin (COO) and M7-FLIPI, respectively, which have clinically established prognostic significance. Furthermore, a dynamic 2-log drop in ctDNA after one round of chemotherapy predicted eventual complete response in DLBCL (p=0.07) and was predictive of both EFS and OS independent of PET/CT (p=0.0015 and 0.0005). We next applied CAPP-Seq to two populations at high risk for developing NHL for which we had prediagnostic blood samples: (1) patients with high circulating t(14;18) levels and (2) heart and lung transplant recipients. We identified tumor-derived genetic aberrations in prediagnostic samples from t(14;18)-positive subjects who subsequently developed FL a mean of 7.8 years prior to diagnosis and in both EBV+ and EBV- PTLD patients a mean of 114 days prior to clinical diagnosis (p<0.03). Finally, we assessed the utility of ctDNA for ultrasensitive detection of minimal residual disease (MRD) and early detection of relapse. We were able to detect ctDNA down to 5 in 1,000,000 cfDNA molecules, allowing detection of MRD a mean of 188 days prior to clinical relapse with 100% specificity.

Conclusions: ctDNA can be used to classify molecular subtypes noninvasively and accurately predict clinical outcomes, potentially guiding future personalized risk-directed treatment. In addition, ctDNA can be used as an effective biomarker for cancer screening and monitoring, which may dramatically improve patient outcomes.
Plasma Soluble CD40 Ligand in Post-Traumatic Systemic Inflammation

David T. Lubkin BS, Mingqing Song PhD, Todd V. Brennan MD, Allan D. Kirk MD, PhD

Background: Major trauma can be followed by overwhelming systemic inflammation, causing vasodilation, increased vascular permeability, and hypercoagulability that can result in hypoperfusion, organ failure, and death. Among trauma patients who survive their initial injuries, this inflammatory response, referred to as systemic inflammatory response syndrome (SIRS), is a major cause of delayed morbidity and mortality. Soluble CD40 ligand (sCD40L) is released by platelets, activated in response to endothelial damage, and may contribute to systemic inflammation through stimulation of CD40 on monocytes and endothelial cells leading to release of interleukin-6 (IL-6), IL-8, and monocyte chemotactic protein 1 (MCP-1). The aim of this study was to investigate the association between plasma levels of sCD40L and the development of post-traumatic SIRS.

Methods: Samples and clinical data were prospectively collected from acutely injured patients as part of the Surgical Critical Care Initiative (SC2i). All samples analyzed were collected within 24 hours of injury. Patients were divided into two groups depending on whether or not they met SIRS criteria within the first three days following injury. Criteria for SIRS were met if a patient simultaneously fulfilled two or more of the following conditions: (1) a body temperature <36 °C or >38 °C, (2) a heart rate >90/min, (3) a respiratory rate >20/min or PaCO2 <32 mmHg, (4) a white blood cell (WBC) count <4000/mm3 or >12000/mm3. Plasma sCD40L levels were measured by enzyme-linked immunosorbent assay (ELISA) and serum IL-6, IL-8, and MCP-1 levels were measured by Luminex® assay.

Results: Of the 29 patients included, 15 (51.7%) developed SIRS within three days of injury and 14 (48.3%) did not. Plasma sCD40L level was significantly higher in the SIRS group than in the no SIRS group [median (IQR) 72.19 (63.78-116.4) pg/ml vs. 55.72 (42.07-77.63), p=0.017]. Serum IL-6, IL-8, and MCP-1 data were available for 25 of the 29 patients (SIRS, n=12; no SIRS, n=13). Median level of IL-6 was significantly higher in the SIRS group [SIRS: 81.92 (42.32-169.4) pg/ml vs. no SIRS: 25.96 (17.19-49.99), p=0.01]. Median levels of IL-8 [46.48 (27.26-99.33) pg/ml vs. 24.27 (8.35-69.09), p=0.12] and MCP-1 [533.9 (418.7-880.6) pg/ml vs. 327.6 (195.4-710.2), p=0.19] trended higher in the SIRS group, but these differences did not reach statistical significance.

Conclusions: sCD40L levels are elevated shortly after injury in patients who develop post-traumatic SIRS and may contribute to this inflammatory response. Elevated sCD40L may be an early biomarker indicating that a patient is at risk for developing SIRS, and CD40 ligand blockade is a potential novel target for prevention and treatment of SIRS.
Validation of a Host Response Signature to Discriminate Bacterial, Viral, and Non-infectious Causes of Illness.

Emily Lydon, Charles Bullard, Mert Aydin, Olga Better, Anna Mazur, Micah T. McClain, Geoffrey S. Ginsburg, Christopher W. Woods, Thomas Burke, Ricardo Henao, Ephraim L. Tsalik Eugene A. Stead Student Research Scholarship, Infectious Diseases Society of America Medical Scholars Program

**Background:** Bacterial and viral infections are difficult to clinically distinguish, leading to antibiotic overuse and resistance. Host gene expression signatures are an alternative to traditional pathogen-detection methods to differentiate these etiologies. Several gene expression signatures have been described although performance in ambiguous clinical phenotypes is unknown. Here, we validate a host response signature in subjects with known etiology and explore performance in microbiology-negative and coinfection cases.

**Methods:** RT-PCR was used to measure 87 gene transcripts in a training cohort of 151 samples from patients with microbiologically confirmed and clinically adjudicated phenotypes (48 bacterial; 54 viral; 49 non-infectious illness). This data was used to construct three distinct classifiers: bacterial vs. non-bacterial; viral vs. non-viral; and non-infectious vs. infectious. This model was then applied to 75 subjects with coinfection and 39 suspected bacterial cases without microbiological confirmation.

**Results:** Leave-one-out cross validation in the training cohort demonstrated AUC values of 0.85, 0.89, and 0.88 for bacterial, viral, and non-infectious illness, respectively. In 39 subjects with suspected bacterial infection but no confirmatory microbiology, a bacterial infection or bacterial/viral coinfection was present in 72%. Among 53 subjects with microbiologically confirmed acute bacterial and viral infection, 95% were identified as having a bacterial or coinfection using gene expression. Among 22 subjects with bacterial infections and chronic viral infection (e.g., HCV, HIV), gene expression identified the bacterial infection in 77%. Procalcitonin performed well in distinguishing bacterial from non-bacterial infection but was unable to identify cases of coinfection.

**Conclusions:** Host gene expression signatures distinguished bacterial, viral, and non-infectious causes of illness. The host response confirmed the majority of suspected bacterial infection without confirmatory microbiology but also indicated a viral etiology in a significant number. Furthermore, the use of distinct viral and bacterial signatures was capable of identifying coinfection better than existing biomarkers. Such a host gene expression strategy, which is being translated to a clinically useful platform, can offer new insights into the etiology of both simple and complex cases that are not currently available.
Utilization of $^{18}$FDG-PET-based IMRT Planning to Spare the Most Metabolically Active Subvolume of Parotid Glands During Definitive Radiotherapy for Head and Neck Cancer

Hue Marley, Yvonne M. Mowery, Qiuwen Wu, David M. Brizel

**Background:** To evaluate $^{18}$FDG-PET-based IMRT planning to reduce radiation dose to the most metabolically active regions of the parotid glands during radiation therapy for head and neck squamous cell carcinoma (HNSCC) with the goal of reducing xerostomia.

**Methods:** Twenty patients with HNSCC (19 oropharynx; 1 nasopharynx) underwent $^{18}$FDG-PET and contrast-enhanced CT simulation for definitive radiation treatment planning. The original IMRT treatment plan used clinically was generated based on the pretreatment CT with volumetric-based dose constraints for each parotid gland. Prescription was 44 or 50 Gy (low risk PTV) and 70 Gy (high risk PTV) at 2 Gy/fraction. Patients were retrospectively replanned with added metabolically-based constraints for each parotid gland. SUV thresholding was performed in Eclipse to generate a contour encompassing the top one-third most metabolically active region of each parotid gland (SUV-High). SUV-High contours were transferred to the registered CT for the replanning. The original IMRT plans were re-optimized to reduce dose to SUV-High within each parotid while maintaining target coverage and meeting initial dose constraints for all other organs at risk. Median doses ($D_{\text{median}}$) to SUV-High and the entire gland for each parotid were calculated for the original and revised plans. Wilcoxon matched-pairs signed rank test (2-tailed, $p<0.05$) was performed to compare $D_{\text{median}}$ in the initial vs. metabolically-based plan for each structure.

**Results:** SUV-High represented a median of 44% (IQR 38.9 – 52.4%) and 40.7% (IQR 21.7 – 54.8%) of the left and right parotid gland volumes, respectively. Median dose differed significantly between the metabolically-based (replan) and clinical (original) IMRT plans for the entire gland and SUV-High for each parotid ($p < 0.0001$). The table shows $D_{\text{median}}$ from each plan, as well as relative percent change in $D_{\text{median}}$ for the metabolically-based plan relative to the original plan for each structure.

**Conclusions:** We have demonstrated that it is feasible to utilize $^{18}$FDG-PET information in IMRT planning to significantly reduce radiation dose to the most metabolically active sub-volume and whole gland for both parotids. Prospective evaluation is necessary to determine whether this approach can reduce late treatment-related xerostomia for head and neck cancer patients.

<table>
<thead>
<tr>
<th></th>
<th>SUV-High, Left Parotid</th>
<th>Whole Gland, Left Parotid</th>
<th>SUV-High, Right Parotid</th>
<th>Whole Gland, Right Parotid</th>
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<tbody>
<tr>
<td><strong>Original $D_{\text{median}}$ (IQR), Gy</strong></td>
<td>14.3 (11.6, 25.8)</td>
<td>16.3 (13.8, 22.3)</td>
<td>18.3 (10, 27.6)</td>
<td>18.8 (14.8, 22.7)</td>
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<tr>
<td><strong>Replan $D_{\text{median}}$ (IQR), Gy</strong></td>
<td>13.7 (10.7, 21.5)</td>
<td>15 (13.2, 20.1)</td>
<td>15.5 (9.3, 24.4)</td>
<td>16.8 (13.9, 19.6)</td>
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<tr>
<td><strong>Median % Change (IQR)</strong></td>
<td>-8.1% (-12.8, -3.9%)</td>
<td>-4.6% (-10.2, 0.1%)</td>
<td>-13.7% (-18, -7.1%)</td>
<td>-6% (-11.8, -0.2%)</td>
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</table>
**Background**: Clinical trial data suggest that diabetes mellitus (DM) is associated with worse outcomes in heart failure with preserved ejection fraction (HFpEF), but data from clinical practice are limited. We hypothesized that patients with HFpEF and DM have distinct clinical characteristics and worse in-hospital and post-discharge outcomes compared to those without DM.

**Methods**: We conducted a retrospective cohort study of 232,656 patients with HFpEF hospitalized for worsening HF in the Get With The Guidelines-HF registry, of which 62,402 were linked with Medicare claims for analysis of post-discharge outcomes. We compared baseline characteristics, in-hospital, and post-discharge outcomes in those with and without DM. Interactions between DM and B-type natriuretic peptide (BNP) and troponin with respect to in-hospital outcomes were evaluated.

**Results**: Patients with HFpEF and DM were more likely to be younger, male, non-white, and to have multiple comorbidities compared with those without DM. Patients with DM had longer length of stay (OR 1.27; 95% CI 1.23-1.31) and decreased likelihood of discharge to home (OR 0.83; 95% CI 0.81-0.86), but there was no association with in-hospital mortality (OR 1.05; 95% CI 0.99-1.11). Similarly, DM was associated with increased 30-day all-cause readmissions (HR 1.10; 95% CI 1.05-1.15) and HF readmissions (HR 1.21; 95% CI 1.12-1.31) but had no effect on 30-day mortality (HR 1.03; 95% CI 0.95-1.11). DM did not affect the association between elevated troponin and worse in-hospital outcomes, but the association between higher b-type natriuretic peptide (BNP) levels and those outcomes was attenuated in those with DM.

**Conclusions**: DM was associated with prolonged hospital stays, decreased likelihood of discharge home and increased likelihood of 30-day readmission without impacting mortality in a large cohort of patients with HFpEF. These findings highlight a critical need for treatment and management strategies for HFpEF and DM, in order to improve quality of life, reduce resource utilization, and slow progression.
Gift Cards for Well Checks: What are Medicaid and CHIP Programs Doing to Incentivize Child Health Behaviors?

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Background: Beneficiary incentive programs, which have become popular in commercial insurance plans, have been increasingly utilized by Medicaid and Children’s Health Insurance Program (CHIP) to promote positive health behaviors (often using rewards). We have previously identified 113 incentive programs run by Medicaid or CHIP agencies or managed care organizations (MCOs). Several states have implemented programs targeting childhood health behaviors and healthcare utilization. We describe the landscape of Medicaid and CHIP beneficiary incentives for children’s health. We also present stakeholder insights on: (1) program design and rationale, (2) engagement of children and families, and (3) program evaluation.

Methods: We conducted an in-depth review of the literature to document a landscape of incentive programs for child health in Medicaid and CHIP. We examined peer-reviewed articles as well as white papers from government and commercial sources, reports on program evaluation, and publicly available beneficiary materials. Informed by our review of the literature, we conducted semi-structured key stakeholder interviews with 80 leaders from state and federal Medicaid offices, MCOs, evaluators, and patient advocates. Specific child-focused insights were collected from 23 stakeholders with child health policy or child incentive program expertise. Interviews focused on program rationale, beneficiary engagement, and program evaluation.

Results: We identified 82 incentive programs targeting children’s health through 1115 waivers, state plan amendments, a federal grant program established by the Affordable Care Act, or MCOs. The most commonly incentivized behaviors were attending well-child checks (n=76), preventive screenings (n=30), and chronic disease management (n=30). Incentives offered included monetary rewards (n=64; gift cards or vouchers) and prizes (n=19; e.g., car seats, bike helmets). Punitive incentives were uncommon (n=1; loss of benefits). Stakeholders found that the rationale for incentivizing child and adult health behaviors were similar (e.g., improved health, meeting quality metrics), though punitive incentives for “personal responsibility” were strongly discouraged for child health. Family engagement was seen as critical but challenging; stakeholders suggested offering incentives in the form of multigenerational rewards (e.g., parent and child), health-promoting prizes (e.g., books, diapers), or services that target the social determinants of health (e.g., transportation, care coordination). Stakeholders suggested incentivizing specific evidence-based preventive services instead of the commonly incentivized well-child check attendance alone and suggested identifying more proximal evaluation measures.

Conclusions: We provide the most comprehensive landscape of Medicaid and CHIP beneficiary incentive programs for children’s health. Limited evidence exists on optimal program design and measuring program impact can be challenging. Stakeholders made several recommendations for the unique opportunities to engage children and families.
US Adolescent and Young Adult Physical Activity Domains: Associations with Weight and Recommendation Adherence, 2007-2016

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**Background:** Youth become less physically active in adolescence and young adulthood, which is an important contributor to the increasing prevalence of obesity. Physical activity can occur in the domains of recreation (leisure-time), occupation (employment, household, or training), and transportation (walking or biking to travel). We examined adolescent and young adult physical activity patterns across these domains, including associations with weight and with rates of meeting physical activity recommendations. We hypothesize that those above a healthy weight will be less likely to perform physical activity in any domain and will be less likely to meet physical activity recommendations than those of healthy weight. We also hypothesize that adding occupational and transportation activity to recreational activity when calculating the total duration of activity will increase the estimated proportion of individuals meeting age-appropriate physical activity recommendations.

**Methods:** We examined 11,157 adolescents and young adults aged 12–29 years reporting physical activity in recreation, occupation, and transportation domains in the 2007–2016 National Health and Nutrition Examination Survey. We compared adjusted odds of performing any physical activity in each domain by weight status. We calculated proportions meeting physical activity recommendations (adolescents: 420 minutes; young adults: 150 minutes) by domain. All estimates are weighted and stratified by age (adolescents: 12–19 years; young adults: 20–29 years) and sex.

**Results:** Most adolescents (90.9%) and young adults (86.7%) reported physical activity in at least one domain. The average portion of an individual’s total physical activity from recreation was 60.2% in adolescents and 42.5% in young adults. Approximately half of young adults reported any occupational activity; occupational activity accounted for 44.6% of total minutes of physical activity among males and 37.4% among females. Weight was inconsistently associated with different physical activity domains; obesity was generally associated with decreased odds of performing physical activity across domains. However, overweight and obesity were generally associated with increased odds of occupational activity. The proportion of adolescents and young adults meeting physical activity recommendations increased when adding domains: recreation alone (34.9% adolescents, 45.6% young adults); recreation and occupation (47.2% adolescents, 68.7% young adults); and recreation, occupation, and transportation (53.5% adolescents, 74.7% young adults) (P < 0.001).

**Conclusions:** Occupational activity represented a significant proportion of total physical activity for adolescents and young adults, particularly among young adult males. Significantly more met physical activity recommendations when adding occupational and transportation activity. Efficient and thorough survey measures and analyses of adolescent and young adult physical activity domains are needed, as comprehensive activity estimates among adolescents and young adults are important to inform public health progress and priorities.
**Immunosuppressive Medications Cause Mitochondrial Dysfunction in T Cells**

Amanda Nash, BS & Todd V Brennan, MD, MS

**Background:** Immunosuppressive medications are widely used for the prevention of allograft rejection in transplantation, as well as for the prevention of autoimmune and graft-vs-host disease. Despite their clinical utility, these medications are accompanied by multiple off-target effects, some of which may be mediated by their effects on mitochondria, which are critically important for T cell function.

**Methods:** We examined the effect of mycophenolate mofetil (MMF), cyclosporine A (CsA), rapamycin, and tacrolimus on mitochondrial function in human T cells. Jurkat cells were cultured in the presence of the above immunosuppressive medications at therapeutic and supra-therapeutic doses. Following incubation, mitochondrial membrane potential, cell death, and superoxide production were measured using flow cytometry. Oxygen consumption rate was measured using a Seahorse bioanalyzer.

**Results:** Following exposure to CsA, T cell mitochondrial membrane depolarization was observed without increased ROS production or apoptosis. MMF induced superoxide production and significant apoptosis at all tested concentrations. MMF and CsA both caused a decrease in basal oxygen consumption rate within the therapeutic range. MMF showed a trend toward decreased ATP-linked respiration without affecting non-mitochondrial respiration.

**Conclusions:** The impairment of mitochondrial function by commonly used immunosuppressive reagents may contribute to the impairment of T cell differentiation and function by decreasing energy production and producing toxic reactive oxygen species.
Predicting Intracranial Progression Following Stereotactic Radiosurgery for Brain Metastases: Implications for post SRS Imaging

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Background: Brain metastases (BM) are a common site of metastatic disease presentation and progression occurring in approximately 20-40% of cancer patients. Stereotactic radiosurgery (SRS) is a standard treatment for patients with limited BM. Follow-up MRI imaging is crucial to identify salvageable intracranial progression. However, optimal imaging intervals are poorly understood in this heterogenous patient population with variable survival. Thus, we sought to build a predictive model for time to intracranial progression.

Methods: Consecutive patients treated with SRS for BM at three institutions from January 1, 2002 to June 30, 2017 were identified. Patient records and intracranial imaging were retrospectively reviewed at each follow-up visit recording symptoms and progression. We developed a model using stepwise regression that identified four prognostic factors from which a clinical nomogram was built to predict time to intracranial progression.

Results: We identified 755 patients, with good performance status (ECOG 0-1: 88%) and primarily non-small cell lung (n=337), breast (n=147), and melanoma (n=129) BMs. Median survival was 12.5 months. Cumulative incidence of symptomatic and asymptomatic intracranial progression at 6 months was 14% and 22%, respectively. The number of treated BMs, tumor histology, history of prior whole-brain radiation, and time interval from initial cancer diagnosis to first metastasis were prognostic for intracranial progression. These factors were used to build a nomogram stratifying patients into high and low-risk cohorts for progression. Those stratified into the high-risk group had a 21% chance of intracranial progression by three months post-SRS compared to 11% in the low-risk group; at 6 months, it was 43% versus 27%.

Conclusions: We present a novel predictive nomogram estimating time to intracranial progression of brain metastases following SRS to provide anticipatory guidance for both patients and clinicians. Ideally, patients with low risk of progression after SRS could be spared the cost and anxiety of undergoing standard surveillance MRI imaging every 3 months. Alternatively, high-risk patients are more likely to rapidly develop new BMs and should be monitored at shorter time intervals. These high-risk patients would otherwise present symptomatically, resulting in increased cost, higher rates of neurosurgical intervention, longer inpatient hospital stays, higher risk of neurological death, and decreased overall survival. Our model seeks to guide individualized surveillance imaging regimens to reduce symptomatic BM progression and its associated quality of life, psychosocial, and financial burden.
Comparison of Programmed Intermittent Epidural Boluses with Continuous Epidural Infusion for the Maintenance of Labor Analgesia: A Randomized Controlled Double-Blind Study

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Background: Programmed Intermittent Epidural Boluses (PIEB) may improve the spread of local anesthetic compared to continuous epidural infusion (CEI) possibly resulting in improved labor analgesia and obstetrical outcomes. Most of the available evidence comes from retrospective studies or studies that predated the availability of pumps capable of co-delivering PIEB with patient controlled epidural analgesia (PCEA). Many earlier studies used two pumps, one to administer PIEB or CEI and one to administer PCEA, or pumps not available in North America. We performed this study to compare PIEB vs CEI for labor analgesia. We hypothesized that PIEB would lower PCEA consumption compared to CEI.

Methods: Women requesting epidural analgesia at 2-7 cm cervical dilatation were enrolled. Analgesia was initiated and maintained with ropivacaine 0.1% with fentanyl 2 mcg/ml. After 20 mL loading dose, patients were randomized to PIEB 6 mL every 45 minutes (first bolus 30 minutes after epidural initiation) or CEI 8mL/h. Randomization was stratified by parity. Patients received 8mL PCEA boluses, with 10-minute lockout period. Patients and providers were blinded to the regimen used. Rescue boluses of 5 mL of 0.2 % ropivacaine were administered for inadequate analgesia. Primary outcome was PCEA consumption/h. Secondary outcomes included need for physician interventions, PCEA attempts, ratio of total/successful PCEA attempts, time to first PCEA attempt, motor blockade, frequency of hypotensive events, pain scores, duration of second stage, mode of delivery, and maternal satisfaction. Continuous data were analyzed with Wilcoxon-Rank or t-test and categorical data with chi-squared or Fisher’s exact test. Pain and Bromage scores were assessed longitudinally between groups with mixed modeling while accounting for patient level clustering.

Results: We included 120 patients, 86 were primaparous. There were no significant differences in patient demographics between the groups. There were also no significant differences between the groups in the primary outcome or any of the secondary outcomes except for a higher PCEA attempts/given ratio/h in the PIEB group (Table). Differences between groups in pain and Bromage scores as repeated measures were insignificant (p= 0.06; 0.81 respectively).

Conclusions: Under the conditions of our study, we did not find improved outcomes with PIEB compared to CEI. Future studies should evaluate different settings of PIEB to optimize analgesia and outcomes.
The Role of the Endothelial Cell Scaffolding Protein Caskin-2 in eNOS Mediated Regulation of Vascular Homeostasis

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The Eugene A. Stead Jr. MD Research Scholarship

Background: Defects in vascular homeostasis are responsible for a variety of cardiovascular disorders that have an impact on public health. To understand the signaling pathways downstream of the Tie receptors, the Kontos lab performed a yeast 2-hybrid screen and identified protein interactors specific for each receptor. One such interactor was identified as Caskin2, a molecular scaffold based on the presence of numerous protein-protein interaction domains. An important regulator of normal endothelial cell function and vascular homeostasis is endothelial nitric oxide synthetase (eNOS). Preliminary data from the Kontos lab has demonstrated that Caskin2 may be a link between eNOS-mediated endothelial homeostasis. Based on these and preliminary studies, we aimed to determine the molecular mechanisms by which Caskin2 regulates eNOS activity and endothelial function in vitro.

Methods: Human umbilical vein endothelial cells (HUVECs) were cultured in endothelial EGM and 293T and BAEC cells were cultured in DMEM. 293T and BAEC cells were transfected with pcDNA3.1-HA-Caskin2-WT/S858A and GFP using Lipofectamine 2000. HUVECs were infected with AdCaskin2 or AdGFP overnight in EGM. For the CamKII assay, each sample was incubated with 50 ng of active CAMKII. HA-tagged proteins were immunoprecipitated by incubating the whole cell lysate (WCL) overnight with Pierce Anti-HA Agarose overnight at 4°C. Samples were prepared for SDS-PAGE and then blotted with p-eNOS (S1177), Caskin-2 (H-7), Calmodulin, Caveolin-1 and Phospho-Akt-substrate antibody.

Results: We observed that an overexpression of AdCaskin-2 in HUVECs increased eNOS phosphorylation on the S1177 residue, but Isoform B or AdGFP did not show an increase in eNOS phosphorylation. In addition to eNOS, Caskin-2 is known to bind to calmodulin (CaM) and caveolin (Cav)-1. There was no differential binding between Caskin-2 WT or Caskin-2 Isoform-B when blotted for Caveolin-1 or Calmodulin. Calcium/calmodulin-dependent protein kinase II (CaMKII) has been shown to phosphorylate eNOS on Ser1177. We observed when comparing WT-Caskin-2 vs. Caskin2-S858A, there was a marked increase in the phosphorylation of eNOS in the wild-type treated groups, but not in the Caskin2-S858A group. However, when CaMKII was replaced with Akt, we observed that those treatment groups lacking the addition of Akt had no phosphorylation activity. Akt potentially plays a necessary role in eNOS phosphorylation at the S1177 residue.

Conclusion: In conclusion, we discovered Caskin-2 plays a role in the phosphorylation of eNOS on the S1177 residue and is regulated in part by CaMKII and Akt. Our initial experiments have provided important insights into the function of this novel protein and its dependence on signaling through eNOS for its functional effects.
Health Insurance Status, Stage at Presentation and Survival Among Female Patients with Head and Neck Cancer


**Background:** Head and neck cancer epidemiology has historically focused on the burden of disease among males. Despite the fact that recent data illustrate a concerning increase in the incidence of certain types of head and neck cancer among females, females with head and neck cancer continue to represent an understudied and overlooked patient population. Previous studies have established that health insurance status is associated with mortality and stage at presentation among patients with head and neck cancer. The impact of health insurance status on female patients with head and neck cancer, however, is not well understood. In an evolving social and political landscape, understanding how historically overlooked populations of cancer patients are impacted by health insurance may help inform how to mitigate existing disparities in care and outcomes. This study evaluates the association between health insurance status, stage at presentation and survival among female patients with head and neck cancer and serves as a call to action for clinicians and epidemiologists to increase knowledge and awareness of the head and neck cancer experience in this population.

**Methods:** The 2007 to 2014 Surveillance, Epidemiology, and End Results (SEER) 18 database from the National Cancer Institute was used to capture female patients diagnosed with a malignant primary head and neck cancer. The database was queried for information on stage at presentation, cause of death, survival, and insurance status, including private insurance, uninsured and Medicaid. Covariates included age at diagnosis, race/ethnicity, marital status, and tumor site. Chi-square and t-tests determined the association between independent variables and stage. Cox and Fine-Gray proportional hazards models were used to examine the impact on overall and head and neck cancer specific mortality.

**Results:** Patients with Medicaid (aOR=1.59, 95% CI 1.45, 1.74) and who were uninsured (aOR=1.73, 95% CI 1.47, 2.04) were more likely to be diagnosed with advanced stage head and neck cancer. Cancers of the hypopharynx (81%) and oropharynx (83%) were most likely to be diagnosed at an advanced stage. Patients with Medicaid (aHR=1.47, 95% CI 1.38, 1.56) and who were uninsured (aHR=1.45, 95% CI 1.29, 1.63) were more likely to die from any cause compared to privately insured patients. Medicaid (aHR=1.34, 95% CI 1.24, 1.44) and uninsured (aHR=1.41, 95% CI 1.24, 1.60) patients also had a greater hazard of death from head and neck cancer compared to privately insured patients. The incidence of stage IV head and neck cancer in this subpopulation rose by 1.24% from 2007-2014 (APC=1.24, 95% CI 0.30, 2.20).

**Conclusions:** This study illustrates the need to evaluate and address the unique burden of head and neck cancer among females. It is critical for otolaryngologists, primary care physicians and gynecologists to be aware of the trends in head and neck cancer among females and the need for further evaluation or referral of their high-risk patients when concerned.
Predicting Hospital Admission at Emergency Department Triage: A Novel Prediction Model

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Duke NUS Fellowship

Background: Emergency department (ED) overcrowding is a growing international patient safety issue. A major contributor to overcrowding is long wait times for inpatient hospital admission. Patient outcomes worsen with increasing wait times. Wait reduction strategies could significantly improve outcomes. One strategy is earlier notification of the admitting ward of likely admissions from the ED via an admission prediction model. A patient with a high probability of admission could trigger an alert to the admitting ward who could prepare the bed while the patient is still in the ED. The objective of this study is to create a model that can predict a patient’s need for hospital admission at the time of triage using demographic, administrative, and clinical information readily available at triage.

Methods: Retrospective observational study of electronic clinical records of all ED visits over ten years to a large urban hospital in Singapore. The data was extracted from a data warehouse compiled from several hospital databases. The data was randomly divided into a derivation set (70%) and a validation set (30%). We used the derivation set to develop a linear regression model that predicts probability of hospital admission for patients presenting to the ED. We tested the model on the validation set and evaluated retrospective performance by measuring the AUC of the ROC curve. The optimal cutoff was chosen to maximize sensitivity and specificity.

Results: A total of 1,232,016 visits were included for final analysis, of which 38.7% were admitted. The derivation set contained 864,246 visits and the validation set contained 370,392. Eight variables were included in the final model: age group, race, postal code, day of week, time of day, triage category, mode of arrival, and fever status. The model performed well on the validation set with an AUC-ROC of 0.825 (95% CI 0.824-0.827). Increasing age, increasing triage acuity, and mode of arrival via private patient transport were most predictive of the need for admission. Patients who had arrived by private transport had an odds ratio of admission of 8+ compared to those arriving by ambulance of 2+. Patients who lived further away from the study site hospital were also more likely to be admitted.

Conclusions: We developed a model that accurately predicts admission for patients presenting to the ED using demographic, administrative, and clinical data routinely collected at triage. Implementation of the model into the electronic health record could help reduce the burden of overcrowding.
**Shifting the Paradigm in Preclinical Cancer Modeling: A Mouse-Dog-Human Personalized Medicine Approach to Identify Novel Therapeutics for Sarcoma**

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*The Eugene A. Stead Jr. MD Research Scholarship*

**Background:** Soft tissue sarcoma and sarcomas of the bone both suffer from low prevalence in human populations, complicating the study of disease. Due to higher prevalence of these cancers in pet dogs, they provide a unique opportunity to serve as an adjunct to human patients. We aim to validate a mouse-dog-human (MDH) personalized medicine pipeline that utilizes comparative oncology to identify novel therapies for potential clinical application.

**Methods:** We obtained tissue from biopsied or resected canine and human sarcoma and generated patient derived xenograft (PDX) mouse models and cell lines. We generated one canine and one human osteosarcoma PDX cell line and mouse model, D418X and 17-3X, respectively. To identify novel therapeutic candidates for osteosarcoma, we performed a pilot drug screen of 119 FDA approved cancer therapeutics in five human and four canine OS cell lines. To identify additional candidate drugs for 17-3X and D418X, we performed an expanded drug screen using the NIH Bioactive 2,100 compound assay. The top drugs identified from the drug screens, bortezomib (proteasome inhibitor) and verdinexor (CRM1 inhibitor), were validated through *in vivo* PDX mouse clinical trials. To test the efficacy of the pipeline for personalized care, we recruited a three-year-old male Golden Retriever who developed seven synchronous leiomyosarcomas (LMS). We generated a PDX mouse and cell line model from one of the seven primary tumors. Through *in vitro* drug screens, we identified alvespimycin (HSP inhibitor) and bortezomib as top candidate drugs that were validated *in vivo.*

**Results:** Results from the 119-drug screen across both human and canine OS cell lines were indistinguishable and identified proteasome inhibitors as top drug candidates. An expanded drug screen with the newly developed D418X and 17-3X PDX cell lines identified proteasome inhibitors and CRM1 inhibitors as candidate drugs for *in vivo* validation. Verdinexor was found to significantly inhibit tumor growth in both human and canine OS PDX mice compared to control tumors treated with DMSO (p<0.05). The canine LMS patient developed metastatic disease during our *in vivo* experiments which showed significant tumor growth inhibition in bortezomib treated mice (p<0.05). The veterinary oncology team elected to utilize this preclinical information to treat the patient with bortezomib for recurrent and metastatic disease. Though the recurrent lesion that received both bortezomib and palliative radiation therapy decreased in size initially, after four weeks of bortezomib, all lesions increased in size and the patient was transitioned to palliative care.

**Conclusion:** Our cross-species MDH pipeline has identified a novel targeted agent, verdinexor, for the treatment of osteosarcoma in both species. Through our canine LMS patient, this study shows that it is feasible to generate patient derived cancer models, identify new therapeutics through *in vitro* and *in vivo* preclinical validation, and utilize that information to guide clinical management when patients have disease relapse.
Improving T cell Activation and Combating Exhaustion Licenses Checkpoint Blockade Against Intracranial Tumors

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Introduction: Checkpoint blockade has ushered in a new era of FDA-approved immunotherapeutic strategies for otherwise terminal cancers; however, this approach has had limited success in the brain. Poor baseline T cell activation compounded by mounting expression of exhausting inhibitory checkpoints on T cells within the intracranial environment are limiting factors previously highlighted by our group. Here, we explore platforms combining potent T cell activators with rational, customized multi-checkpoint blockade in murine glioma and brain metastasis models.

Methods: Previously characterized, highly expressed classical and alternative inhibitory checkpoints were selected for targeting (PD-1, TIM-3, LAG-3, TIGIT). A mouse agonist antibody to 4-1BB (CD137) was utilized as T cell activating therapy. Mice were implanted with intracranial CT2A glioma or Lewis Lung Carcinoma (LLC), then received intraperitoneal treatments with respective antibodies. Kaplan-Meier survival curves were generated. Flow cytometry was utilized to characterize tumor-infiltrating lymphocytes (TILs).

Results: TILs from CT2A expressed high levels of 4-1BB, while TILs from LLC did not. Likewise, in the CT2A glioma model, 4-1BB agonism in combination with anti-PD-1 demonstrated a synergistic effect, with 50% long-term survival, where PD-1 blockade alone was entirely ineffective. Combinations of anti-PD-1/TIM-3/LAG-3/TIGIT with 4-1BB agonism resulted in an unprecedented long-term survival rate of 100%, with survivors universally rejecting re-challenge with tumor. Targeting multiple checkpoints in the absence of 4-1BB agonism produced no survival benefit. These strategies demonstrated limited efficacy in LLC, where 4-1BB was poorly expressed.

Conclusions: Poor T cell activation and the up-regulation of multiple classical and alternative immune checkpoints on T cells are limiting factors for checkpoint blockade in the intracranial environment. Customized targeting of all highly expressed checkpoints, in combination with 4-1BB agonism, may obviate these limitations and license checkpoint blockade therapy. Such strategies may also depend on the presence of 4-1BB on T-cells as a target for agonism.
The Civil War Soldier as the Model for Treating Hysterical Women in the 19th Century

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Trent Center for Bioethics, Humanities & History of Medicine Award

Background: William Hammond (August 28th, 1828 – January 5th, 1900) and Silas Weir Mitchell (February 15th, 1829 – January 4th, 1914) established the specialty of American neurology in the late 19th century. Though hysteria was the hallmark diagnosis of the time and the rest cure an important therapeutic intervention, historians tend to gloss over American neurology and its innovations as derivative of the more famous and previously established European models of neurology. However, American neurology was different, largely because of its roots in Civil War medicine. Both physicians used their training in the Army, Dr. Hammond as the United States Surgeon General and Dr. Mitchell as a contract surgeon, as a basis for modeling their careers and research. This project sought to explain the links between a pivotal period in American history with the medicine that serves as the basis for American neurology using the physicians’ own words and bodies of correspondence.

Methods: I researched first edition publications and handwritten primary sources, including correspondence from William Hammond and Silas Weir Mitchell and their personal case notes. The main source of materials was Duke University’s Rubenstein Rare Book and Manuscript Collection. The College of Physicians in Philadelphia library, Jefferson University library, and University of Pennsylvania Kislak Center for Special Collections, Rare Books, and Manuscripts were consulted for their materials concerning the Civil War, hysteria, 19th century neurology, and the physicians explored in this research. Three graduate courses supplemented this independent study. Dr. Freeman’s Shamanism course in Anthropology provided a framework for studying non-Western European perceptions of many of the symptoms ascribed to hysteria. Magic, Science, and Religion Since 1400 in the History Department with Dr. Robisheaux focused on the philosophies and cultural forces spurring scientific and religious development in Western Europe. The yearlong independent study of 19th Century Neurology with Dr. Humphreys covered the biographical study of the founding fathers of modern neurology, including Jean-Marie Charcot, William Hammond, and Silas Weir Mitchell. The tension between American neurology and the developing fields of psychology and psychiatry in asylums was explored, especially respective methods of parsing truth from falsehood and eccentricity from insanity.

Results & Conclusions: Drs. Hammond and Mitchell, like many in the 19th century, focused much of their professional energy tackling the most vexing diagnosis of their time: Hysteria. Before treating the upper echelons of New York society women for hysteria, Dr. Hammond cemented neurology in America as a legitimate method of combining the anatomical with the psychological as a professional witness in highly publicized court trials on insanity. Mitchell’s research on hysteria is used as an example of a prominent American neurologist attacking one of the major neurological concerns of the 19th century via his personal service in the Union Army with soldiers suffering from “acute exhaustion,” today often compared to modern Post Traumatic Stress Disorder. This paper demonstrates that wartime experiences unique to the United States allowed American neurology to forge its own path separate from the European model.
Building a Quality Improvement Model: Assessing and Optimizing Concordance of PI-RADS v2 with MRI-Ultrasound Fusion Biopsy

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Background: There has been a surge in use of multiparametric MRI (mpMRI) and MRI-ultrasound (US) fusion biopsy in guiding diagnosis of prostate cancer (PCa). Prostate Imaging Reporting and Data System version 2 (PI-RADS v2) is in use worldwide for standardization of mpMRI reporting. However, there is limited data on “ideal” positive predictive value (PPV) for each PI-RADS v2 category, and there are not clear guidelines on how to assess concordance and discordance between mpMRI and fusion biopsy. Our primary objective is to assess concordance of PI-RADS v2 with MRI-US fusion biopsy with aim towards designing a quality improvement (QI) roadmap that can be more universally applied.

Methods: In this retrospective, HIPAA-compliant study, 73 patients who underwent mpMRI at 3 Tesla with endorectal coil followed by a mpMRI-US fusion biopsy were included. PI-RADS v2 scores were assessed on clinical mpMRI interpretation and a total of 90 lesions included. Detailed pathologic biopsy results were reviewed and assigned a Prognostic Grade Group (PGG). Positive predictive value (PPV) of PI-RADS v2 was calculated for all PCa and clinically significant cancer (CSC) (defined in PI-RADS v2 as ≥ PGG2). Subset analysis of discordant cases was performed by board-certified radiologist with expertise in prostate mpMRI in order to determine need for re-biopsy as part of building a quality improvement framework.

Results: 31/90 (34%), 50/90 (56%), and 9/90 (10%) lesions were PI-RADS 5, 4, and 3, respectively. Positive predictive value (PPV) of PI-RADS 5, 4, and 3 for all PCa is 0.90, 0.54, and 0.33, respectively. PPV of PI-RADS 5, 4, and 3 for CSC is 0.71, 0.34, and 0.11, respectively. Subset analysis of discordant biopsy results for PI-RADS 5 lesions in which CSC was not identified (n=9) revealed true discordant pathology (n=4), mpMRI misclassification of lesions as PI-RADS 5 versus PI-RADS 3 due to size > 1.5 cm but without abnormal DWI findings (n=4), and pitfall of mistaking low signal central zone as PCa (n=1).

Conclusion: PI-RADS v2 has very high PPV for PCa detection for PI-RADS 5 lesions. As expected, PPV decreases slightly for detection of CSC compared to all PCa. Given the high PPV of PI-RADS 5 lesions, those without CSC on fusion biopsy require further analysis as establishing need for re-biopsy is critical in driving meaningful PCa quality improvement. To continue adoption of mpMRI worldwide, more data is needed on the “ideal” PPV for PI-RADS categories as well as algorithms for assessing and monitoring biopsy concordance and discordance, similar to systems used in breast imaging practices.
Geospatial Analysis of Risk Factors Contributing to Loss to Follow-Up in Cleft Lip/Palate Care

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NIH T32 Research Training Award

Background: Cleft lip and/or palate (CL/P) is the most common congenital craniofacial anomaly. Due to its complexity, CL/P is best treated by a multidisciplinary team dedicated to cleft care. Team-based care depends on follow-up at specified time points to monitor and address functional or aesthetic concerns that may arise during a child's development. However, there are a limited number of approved cleft teams in the United States; consequently, some patients who begin care in infancy may be lost to follow-up. The reasons for loss to follow-up (LTFU) are complex but ultimately lead to missed opportunities for essential therapeutic and surgical intervention. This study explores clinical, demographic, and geographic determinants of LTFU in cleft care.

Methods: Medical records were retrospectively evaluated for 558 children with CL/P diagnosed and treated at Duke Children’s Hospital from 1998-2013. The primary outcome was LTFU, defined as three consecutive missed appointments or two years without seeing the cleft team despite attempts at reestablishing follow-up. Patients who transferred care to other teams were not considered LTFU. Spatial dependency of our primary outcome, LTFU, was evaluated using empirical variograms. The probability of LTFU was assessed using a Bayesian approach to hierarchical generalized linear geostatistical modeling. Risk maps were plotted to identify vulnerable communities within North Carolina.

Results: 29% of patients seen in this time period were lost to follow-up. When ignoring spatial dependency, shorter duration of team care was a strong predictor of LTFU (p<0.0001), while socioeconomic status (SES) and cleft phenotype were weakly associated with LTFU. When including spatial dependency in the model, both SES and phenotype became significant predictors of LTFU. Distance from the team and rural/urban designation were not predictive of LTFU. Cartographic representation of predicted probability of LTFU revealed discrete pockets of at-risk communities across North Carolina. Surprisingly, not all of these communities were far from our team; rather, several vulnerable communities were identified in the immediate vicinity of the Duke Children’s Hospital Cleft Team.

Conclusions: Socioeconomic status, duration of team care, and cleft phenotype are associated with the risk of LTFU among cleft patients. Additionally, these factors are spatially dependent. Geospatial mapping revealed specific communities that are high-risk for LTFU across North Carolina and in close proximity to our cleft team. Thus, geostatistical methods are able to identify risk factors missed by traditional statistical approaches and have wide applicability across medical and surgical domains. In future work, we plan to expand this analysis to include patients from all approved cleft teams in North Carolina and the Mid-Atlantic States region. This information will help cleft teams better allocate resources to high-risk areas so that deficiencies in care may be prevented or rectified.
Investigating a Murine Form of Olaratumab as a Radiosensitizer in Sarcomas

Erin J. Song, Kathleen A. Ashcraft, Caitlin Lowery, Yvonne Mowery, Yan Ma, Lixia Luo, Lorraine Campos, Diana Cardona, Louis Stancato, David G. Kirsch

Background: Soft tissue sarcomas (STS) are a heterogeneous group of mesenchymal neoplasms. Prognosis is poor, with overall survival of 12-18 months in metastatic disease. Management often involves a multidisciplinary approach of surgery, radiation, and chemotherapy. Currently, few systemic therapies exist. Since 1975, doxorubicin has been the cornerstone of chemotherapy, both as monotherapy and combined with other agents. However, no agent showed significantly improved survival until the recent phase Ib/II study in which doxorubicin was combined with olaratumab, an antibody against platelet derived growth factor receptor alpha (PDGFRα). This study supported accelerated FDA approval of olaratumab with doxorubicin for metastatic STS. Although radiation therapy (RT) is often used as neoadjuvant treatment, olaratumab has not yet been tested with RT. This work evaluates 1E10, a murine form of olaratumab, as a radiosensitizer in a primary mouse model of STS.

Methods: Primary sarcomas were generated in mice with both copies of p53 flanked by flippase recombinase target sequences (p53FRT/FRT). Upon injection of an adenovirus expressing flippase recombinase, both alleles of p53 were deleted and further mutations induced with the carcinogen 3-methylcholanthrene. As tumors reached 70 mm³, mice were randomized to treatment groups (n=20/group): 1) isotype control, 2) 1E10, 3) isotype + RT, or 4) 1E10 + RT. Intraperitoneal isotype or 1E10 was given biweekly. On the first day of drug treatment, mice in the RT groups also began a protocol for 25 Gy to the tumor (given in five fractions of 5 Gy). To quantify growth delay, time to tumor volume quintupling was measured. A two-way ANOVA was used to assess for differences in response to 1E10 alone, RT alone, or interaction of 1E10 + RT. For histology, tumors were stained for CD31 and PDGFRα via immunohistochemistry, and lungs were assessed for presence of micrometastasis.

Results: We report a significant difference in time to tumor size quintupling between the RT and non-RT groups (p < 0.0001); no difference is seen between isotype and 1E10 groups regardless of RT. Staining for CD31 demonstrated a lower microvessel density in tumors treated with 1E10 + RT, while PDGFRα expression did not vary among groups. Interim analysis of lung samples showed micrometastasis in 3 mice treated with isotype (n=14), 2 mice treated with isotype + RT (n=12), 1 mouse treated with 1E10 (n=10), and none of the mice treated with 1E10 + RT (n=10).

Conclusions: 1E10 did not act as a radiosensitizer in this model of primary STS. Although microvessel density was decreased in tumors treated with 1E10 + RT, this did not affect tumor growth delay. Analysis of lung samples is ongoing, but interim data shows a possibly decreased rate of micrometastasis in 1E10 + RT treated mice.
Adipose-Derived Stem Cells Can Contribute to Vascular Network Formation in Poly(ethylene glycol) Hydrogel Scaffolds

Visakha Suresh, BSE, and Jennifer L. West, PhD

**Background:** Although adipose-derived stem cells (ADSCs) can influence wound healing, their role in neovascularization is unclear. Utilizing three-dimensional in vitro models may provide insight into the in vivo cell-cell interactions between ADSCs and vasculature. To study the relationships between ADSCs, endothelial cells, and pericytes, we seeded these cell types within a peptide-modified poly(ethylene-glycol) (PEG) hydrogel. This 3D cell-adhesive, proteolytically degradable cell culture matrix supports formation of vascular networks by encapsulated endothelial cells and pericytes.

**Methods:** For this study, ADSCs were cultured in either basal media (basal) or EGM-2 media with 20 ng/mL of VEGF (stimulated) and then encapsulated with human umbilical vein endothelial cells (HUVECs) or human brain vascular pericytes (HBVPs). These studies were repeated with diabetic ADSCs to examine the influence of this phenotype on the cells’ ability to influence neovascularization.

**Results:** In co-culture with HUVECs, basal and stimulated ADSCs were capable of enhancing formation of CD31+ tubule-like networks. However, only co-cultures of stimulated ADSCs/HUVECs resulted in tubule diameters comparable to HUVEC/HBVP controls (26.9 ± 1.8 vs. 26.7 ± 0.9 µm, p = 0.87) (Figure 1). When encapsulated with HBVPs, both basal and stimulated ADSCs were capable of forming CD31+ tubule-like networks. Only co-cultures of stimulated ADSCs/HBVPs resulted in tubules diameters comparable to controls (27.6 ± 0.8 vs. 26.7 ± 0.9 µm, p = 0.09).

**Conclusions:** We demonstrated that ADSCs can not only serve as support cells for vascular network formation but also take on an endothelial-like phenotype and form CD31+ vascular structures when encapsulated with support cells. Furthermore, we found that ADSCs from diabetic donors are less capable of serving as a support cell for vascularization, although this functionality can be rescued through culture in a pro-angiogenic medium. Finally, we found that the capability for diabetic ADSCs to take on a functional endothelial-like phenotype is diminished, as evidenced by the lack of CD31 positive vascular network formation when co-cultured with other mesenchymal support cells. Our PEG-based hydrogel served as a highly controlled, tunable system in which to study the specific cell-cell interactions between ADSCs, endothelial cells, and mesenchymal support cells.
The Story in the Numbers:  
Understanding the Effectiveness of Current Cost-Reduction Measures

Aarti Thakkar, Kevin Shaw

**Background**: The Center for Medicare and Medicaid Services (CMS) has prioritized the implementation of innovative programs incentivizing health systems to decrease health care costs while maintaining and improving quality of care for patients. The Medicare Shared Savings Program (MSSP) was introduced as one such cost-containment initiative. The goal of the MSSP is to highlight and identify areas for improvement through extensive data collection of quality metrics as well as patient cost data. However, even with this information, there is uncertainty about how to best utilize this data.

**Methods**: We evaluated two perspectives to approach how to reduce cost amongst MSSP patients. The first is a patient focused approach that emphasizes reducing utilization for patients at high risk for emergency department (ED) or inpatient (IP) visits. The second is a physician focused approach with a goal to change physician prescribing behavior across the system. We used Duke Primary Care and MSSP data from the 2017 fiscal year in order to determine which approach could most efficiently reduce overall costs for MSSP patient. Data was compiled from two sources: (1) The Physician Opportunity Panel Report included all MSSP information from all practices including basic demographics, Medicare costs, ED visits and inpatient visits. (2) Duke Primary Care Panel Report included active numbers of all patients, providers, and payers. The patient-focused approach estimated additional costs of a care-management team at approximately $250,000 a year, combined with actual ED and IP utilization rates. The physician-focused approach used available DUH imaging cost data.

**Results**: We pulled data from 25 Duke Primary Care practices consisting of 184 providers with 21,474 with available cost data. The average cost per patient for all of DPC was $10,323.04 with a median of $3,362.98. The patients in the top 20th cost percentile account for 74% of all costs. The 914 patients identified as high risk accounted for 22% of total costs. Cost reduction analysis revealed that from a patient focused approach, a 2.5% drop would require practices to cut ED visits by high-risk patients by as much as 54% or in-patient visits by 59%. On the other hand, a single physician-centered approach would ask physicians to reduce CT imaging by 60% across the practice.

**Conclusions**: Our data supports the underlying concern that a small number of patients make up a large proportion of total costs. However, the cost reduction analyses reveal that neither a single patient-focused approach nor a single physician-focused approach would be sufficient or even probable to achieve the cost reduction measures proposed. Instead, our analysis supports the use of an integrated system that links patient-centered programs such as care-management with physician engagement and practices in order to enact change for the patient as well as the greater health system.
Development of Intraoperative Topography Maps with Microscope-Integrated Optical Coherence Tomography (MIOCT)

James Tian, Brenton Keller, Nicole M. Fuerst, Ryan McNabb, Melissa Daluvoy, Cynthia Toth, Joseph Izatt, Anthony Kuo

NIH R01-EY023039, R01-EY024312, and RPB departmental grant

Background: Full thickness corneal transplantation is the most commonly performed transplantation procedure in the world, performed for many debilitating diseases such as infectious keratitis, keratoconus, or corneal hydrops. However, asymmetric tension from sutures placed during the transplantation can result in significant post-operative astigmatism, where different parts of the cornea have different curvatures, which greatly reduces the quality of vision. Thus we propose the development of MIOCT topography maps, which can display the astigmatism to the surgeon intraoperatively, such that they could potentially adjust sutures at the point of care to reduce post-operative astigmatism. This an exciting new use of the MIOCT intraoperative visualization system, providing higher order intraoperative analysis beyond merely viewing anatomy.

Methods: A processing pipeline was developed to automatically segment the elevation from MIOCT corneal images, correct image warp and tilt, perform Zernike fitting, and generate topography maps intraoperatively. This was validated on digital and plastic corneas of known curvature and tested in the operating room on a corneal transplant case series.

Results: Validation on digital and plastic corneas generated topography maps with an average curvature within 2 diopters of the true reference curvatures and angle of steep/flat axes within 5° of the true reference angles. Topography maps were generated intraoperatively that showed similar mean curvature values as post-operative day one clinical topography.

Conclusions: We were able to generate topography maps were generated intraoperatively from MIOCT despite high corneal irregularity. Mean curvatures from intraoperative topography were comparable to mean post-op curvatures. This has promise for intraoperative astigmatism management in corneal transplantation and is an exciting new quantitative application of intraoperative imaging.
JAG1 and IL-13Rα2 as Novel Biomarkers within the Lung Cancer Metastatic Invasion Unit

Rachel Tobin, Janna K. Mouw, Ph.D., Emily Summerbell, Jamie Arnst, Ph.D., Adam Marcus, Ph.D.

**Background:** Lung cancer is the leading cause of cancer death worldwide, with many cases presenting as advanced stage disease with widespread metastases. Metastasis in lung cancer has been shown to occur via collective invasion, with heterogeneous populations in the tumor cooperating to promote metastasis. Previously, the Marcus lab used an image-guided genomics approach termed spatiotemporal genomic and cellular analysis (SaGA) to analyze the molecular profiles of two unique phenotypes in the collective invasion pack: leader and follower cells. Leader cells initiate invasion by leading an invasive chain. Follower cells promote survival and proliferation of leader cells. Genomic data of leader and follower cells was analyzed to identify cell surface biomarkers of these invasive subpopulations. Jagged1 (JAG1), a ligand for the Notch signaling pathway, was chosen as a biomarker candidate for leader cells. IL-13 receptor alpha 2 (IL-13Rα2), a receptor for IL-13, was chosen for follower cells. The purpose of this study was to determine if JAG1 and IL-13Rα2 serve as biomarkers of leader and follower cells respectively and influence invasive phenotypes.

**Methods:** cBioportal datasets were analyzed for biomarker mRNA expression and patient survival outcomes. JAG1 and IL-13Rα2 expression was characterized in lung cancer cell lines via flow cytometry, and subpopulations were sorted using fluorescence-activated cell sorting (FACS). Biomarker protein levels were measured using Western blotting. Spheroid invasion assays were used to investigate invasive phenotypes *in vitro* by measuring invasive area and circularity. Functional significance of biomarker expression was further investigated by cell cycle analysis, Celltracker™ staining, morphology analysis, and IL-13 cytokine treatment.

**Results:** Our data demonstrated that in the H1299 cell line, JAG1+ and leader cells demonstrated a similar chain-like invasion pattern, whereas IL-13Rα2+ and follower cells both expressed a sheet-like invasive phenotype. JAG1 and IL-13Rα2 were present in several other lung cancer cell lines, and invasion was significantly increased in JAG1+ H23 cells and H1792 IL-13Rα2+ cells. In a patient derived organoid, JAG1+ cells were significantly more invasive than the parental sample.

**Conclusions:** Results from several lung cancer cell lines and a patient sample supported the hypothesis that JAG1 and IL-13Rα2 serve as relevant biomarkers for leader and follower cells respectively, which could serve as therapeutic targets and provide prognostic information by identifying invasive subpopulations.
Feasibility of Cancer Clinical Trial Enrollment Goals Based on Cancer Incidence

George Tran; Matthew Harker, MPH, MBA; Karen Chiswell, PhD; Joseph M. Unger, PhD; Mark Fleury, PhD; Bradford Hirsch, MD, MBA; Kimberly Miller, MPH; Philip d'Almada, MS; Sheri Tibbs; S. Yousuf Zafar, MD, MHS

**Background:** More than 20% of US clinical trials fail to accrue sufficient patients and terminate prematurely, impeding innovation and negating the valuable contributions of participating patients. The aim of this study is to estimate availability of patients for each trial opening in the national oncology clinical research portfolio to provide a benchmark for better understanding feasibility of clinical trial enrollment goals.

**Methods:** The Database for Aggregate Analysis of ClinicalTrials.gov, up-to-date as of September 3, 2017, was used to identify actively-recruiting, interventional oncology trials at US sites. Observational studies were excluded as not all are registered. Trials were categorized via Medical Subject Headings or free text condition terms and sorted by cancer diagnosis. Trial slot availability was estimated between September 1, 2017, to August 31, 2018. Availability was estimated from total anticipated enrollment, assuming a constant recruitment rate. Estimates for studies with both foreign and US sites were pro-rated to calculate available enrollment in the US alone. The 2017 American Cancer Society cancer incidence estimates were used to approximate total US cancer diagnoses.

**Results:** 4598 oncology trials were identified. Overall, an estimated 12.6 cancer patients are available for each clinical trial slot. The estimates by cancer diagnosis were: colorectal: 24.7 patients per trial slot; lung & bronchus: 20.1; prostate: 17.6; breast (female): 13.8; leukemia 11.6; and brain & other nervous system: 6.0.

**Conclusions:** Across all diagnoses, 1 in 13 patients must enroll to meet accrual demands. This ratio varies by diagnosis. If cancer incidence is too low, trials with unrealistic accrual goals may be doomed at inception. In diagnoses with high disease burden, trial failure may be due to poor patient access or suboptimal design.

<table>
<thead>
<tr>
<th>Cancer diagnosis</th>
<th>Interventional enrollment slots, pro-rated for US sites</th>
<th>2017 Estimated Incidence</th>
<th>Estimated number of incident cancers for each enrollment slot</th>
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<tr>
<td>All Sites</td>
<td>134339</td>
<td>1688780</td>
<td>12.6</td>
</tr>
<tr>
<td>Breast (female)</td>
<td>18294</td>
<td>252710</td>
<td>13.8</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>11077</td>
<td>222500</td>
<td>20.1</td>
</tr>
<tr>
<td>Prostate</td>
<td>9163</td>
<td>161360</td>
<td>17.6</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
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<td>135430</td>
<td>24.7</td>
</tr>
<tr>
<td>Leukemia</td>
<td>5366</td>
<td>62130</td>
<td>11.6</td>
</tr>
<tr>
<td>Brain &amp; other nervous system</td>
<td>3952</td>
<td>23800</td>
<td>6.0</td>
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</table>
Long-term Outcomes and Dynamic Imaging Response for Image-Guided Stereotactic Radiosurgery of Brain Arteriovenous Malformations


**Background:** While fixed head-frame stereotactic radiosurgery (SRS) is an established treatment strategy for arteriovenous malformation (AVM), the safety and efficacy of image-guided SRS (IG-SRS) with relocatable masks is less well-characterized. This study analyzes our long-term experience with IG-SRS in AVM, examining patient and treatment factors, outcome and serial magnetic resonance imaging (MRI) findings.

**Methods:** In this IRB-approved study, we reviewed all charts of patients with brain AVM who underwent IG-SRS at our institution from March 2008 - July 2017. All patients were treated on a linear accelerator with a micro-multileaf collimator and orthogonal kV/cone-beam CT imaging using a relocatable face mask. Planning target volume was the nidus volume contoured on T2-weighted MRI expanded 1mm. Patients received follow up MRI’s 6 months post-SRS and yearly, thereafter. Obliteration was determined via MRI or, preferably, angiography. Adverse events (AE) were determined using CTCAE v4.0. Survival curves were generated via the Kaplan-Meier method. In analyzing dose-volume effect, the LQ model with an α/β of 3 Gy was used to calculate biologically equivalent doses.

**Results:** 71 patients with adequate information available for analysis were identified. Median patient age at SRS was 41 years old (range 4-80) and 28 (39.4%) were female. 35 (49.3%) patients had history of pre-SRS hemorrhage. 40 (56.3%) patients had undergone prior embolization, surgery, or SRS. Median pre-SRS Karnofsky Performance Scale (KPS) was 90 with 6 patients having KPS ≤60. 56 (78.9%) patients had AVMs in eloquent locations. Median nidal volume was 1.6 ml (range, 0.1-36.9) and median prescribed marginal total dose was 1800 cGy (1200-3000). 57 (80.3%) patients underwent single fraction SRS, 12 (16.9%) had 2-fraction SRS and 2 (2.8%) received five 6 Gy fractions. The median volume of brain receiving 10 Gy was 15.9 ml (2.8-160), 12 Gy was 10.8 ml (2.1-115), and 15 Gy was 6.6 ml (0-85.5). Median follow-up was 44.2 months (95% CI 34.2, 56.2). The median time to obliteration was 53.9 months (95% CI 46, 73.3); at 7 years post-SRS, the actuarial obliteration rate was 82.6% (95% CI 60.9, 96.2%). Only 13 patients had severe (≥ grade 3) SRS-related AE; 4 Grade-3, 6 Grade-4, and 3 Grade-5. On univariate analysis, there was no significant association between grade ≥3 AE and irradiated volume, AVM volume or location eloquence. Post-SRS hemorrhage was observed in 6 (8.5%) patients. Overall survival was 91% 5 years post SRS, with 3 patients each dying from causes unrelated to AVM. Of 57 patients with >1 follow-up MRI, 37 (64.9%) saw an increase in T1+C volume with median time to peak volume of 11.1 months (3.1-47.7); 47 (82.5%) patients showed an increase in T2 volume with median time to peak volume of 15.5 months (2.7-78.3).

**Conclusion:** IG-SRS appears to be a safe and efficacious method for treatment of brain AVMs. It is important to recognize that most patients experience post-SRS brain changes that seem to peak about one year following SRS. During this time of evolving brain response, patients require thorough and regular follow-up.
Evidence Against Routine Genetic Testing for Steroid-resistant Nephrotic Syndrome in an Outbred Population

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Doris Duke Clinical Research Mentorship Award

Background: Nephrotic syndrome (NS) is a glomerular disease characterized by massive proteinuria, hypoalbuminemia, hyperlipidemia, and peripheral edema. Steroid-resistant nephrotic syndrome (SRNS) affects approximately 20% of children and young adults with NS and is a leading cause of end-stage kidney disease. Advances in genomics have led to the identification of more than 50 genes associated with SRNS and elucidated podocyte dysfunction as central to its pathogenesis. Although some groups advocate for routine genetic testing in all patients with SRNS, there are currently no clear, evidence-based guidelines for genetic screening in patients with SRNS in clinical practice.

Methods: We identified 492 individuals from 181 nonconsanguineous families with SRNS in this worldwide multi-ethnic cohort. Eighty-six families had familial disease, including 65 with an autosomal dominant pattern of inheritance and 21 with an autosomal recessive pattern. Ninety-five individuals had sporadic disease. Eligible subjects were screened by whole-exome sequencing, targeted sequencing of custom amplicons (TSCA) of 40 known SRNS genes, or direct candidate gene sequencing. Causative variants were defined as missense, truncating, or obligatory splice site variants with a minor allele frequency <1% in the normal population that were found to be pathogenic by at least two in silico software models. In order to provide clinical guidelines, we evaluated for differences in sex, race, age at disease onset, renal biopsy findings, family history of SRNS or chronic kidney disease (CKD), and extrarenal manifestations suggestive of syndromic disease.

Results: We identified 36 unique disease-causing variants in 40 of 181 families (22.1%) with SRNS, 19 (52.8%) of which were novel. Novel variants identified by TSCA included truncating variants in the autosomal dominant genes INF2 (p.E249X) and TRPC6 (p.G39fsX41) and two variants in exon 7 of WT1 (p.C181Y and p.T204A). Variants in INF2, COL4A3, and WT1 were the most common, accounting for over half of all causative variants. Causative variants were identified in 34 of 86 families (39.5%) with familial disease and 6 of 95 individuals (6.3%) with sporadic disease. Family history of SRNS or CKD was the only significant clinical factor predictive of identifying a causative variant ($\chi^2$ p<0.00001).

Conclusions: In this international cohort, we identified single gene causes of disease in almost 40% of families with hereditary SRNS, compared to only 6% of individuals with sporadic disease, making family history the single most important clinical predictor of monogenic SRNS. Based on these data, genetic testing is recommended for patients with SRNS who have a positive family history, but selective testing should be employed for those with sporadic disease.
Optimization of Storage and Transportation Conditions for Cultured Corneal Endothelial Cells

Stephen Wahlig, Gary S. L. Peh, Matthew Lovatt, Khadijah Adnan, Shu-Jun Lin, Xin- Yi Seah, Heng-Pei Ang, Chan N. Lwin, Jodhbir S. Mehta
Duke-Singapore Student Scholar Fellowship

Background: Endothelial dysfunction, whether induced by infection, inflammation, trauma, or genetic dystrophy, is one of the most common causes of corneal blindness. Currently, allogeneic transplantation is the only definitive treatment for corneal endothelial pathology, which is limited by the number of available cadaveric donor corneas. Cellular therapies, which use cadaveric endothelial cells that have been cultured in vitro, have the potential to treat up to 90 patients per donor cornea pair. While recent clinical trial results of this approach have been promising, there is still no viable approach for transportation of these cultured cells from the laboratory to distant clinical sites. While endothelial cells are currently stored in a 5% CO₂, 37°C incubator in the laboratory, these conditions cannot be replicated during transportation. This project aims to develop a hypothermic storage protocol, in which endothelial cells can be preserved at 4°C or 23°C (ambient temperature) to ensure compatibility with pre-existing shipping logistics.

Methods: Human corneal endothelial cells (HCEncs) were exposed to various temperatures (4°C, 23°C, and 37°C) in both adherent and suspension storage models. Optimal storage media and maximal storage duration was selected through evaluation of post-storage viability with calcein AM and Annexin V/PI-based assays. Cellular morphology was assessed with phase-contrast microscopy throughout the preservation process. Following storage and subsequent recovery at 37°C, cell phenotype was assessed with immunofluorescence, determination of gene and protein expression, and proliferative capacity analysis.

Results: Endothelial-SFM was determined to be superior to Optisol-GS as a storage medium at both 4°C and 23°C. HCEnc viability was preserved after up to 8 days in adherent storage, although cellular morphology became grossly abnormal after 4 days at 4°C or 23°C. These morphological changes could be reversed with recovery at 37°C. In suspension storage, HCEncs demonstrated optimal survival at 4°C compared to 23°C. Characterization of post-storage cells demonstrated preserved proliferative capacity and expression of endothelial markers such as Na⁺/K⁺ ATPase, ZO-1, CD166, and cell surface Prdx6 comparable to controls at 37°C.

Conclusions: Hypothermic preservation of HCEncs at 23°C for adherent storage or 4°C for both adherent and suspension storage appears to be a viable strategy. As HCEnc cellular therapies emerge as promising clinical therapies, this method of preservation may be critical for transportation of cells from the laboratory to the surgeon.
Obesity is Associated with Increased Prevalence of Glenohumeral Osteoarthritis and Arthroplasty

Kevin C. Wall, BA, Cary S. Politzer, MD, Grant E. Garrigues, MD

Background: Obesity and osteoarthritis are two interrelated and highly prevalent medical conditions that have wide ranging influences, but their relationship to shoulder function is understudied and underappreciated. Recent literature suggests a role of adipokines, adipose tissue-derived cytokines that are at increased circulating levels in higher body mass index (BMI) individuals, in cartilage degeneration, thus providing a plausible link between BMI and glenohumeral osteoarthritis. The aim of this study was to examine the association between BMI and the prevalence of glenohumeral osteoarthritis and arthroplasty.

Methods: This cohort study compared 596,856 age and gender matched patients from a Humana database across six evenly-distributed BMI cohorts. Glenohumeral osteoarthritis prevalence was determined in each cohort, as was a standardized arthroplasty rate by dividing the number of shoulder arthroplasties performed in a cohort by the number of patients who have glenohumeral osteoarthritis in that cohort. Odds ratios for osteoarthritis and arthroplasty rates were found by comparing the reference, BMI under-19, cohort to the other five cohorts.

Results: Glenohumeral osteoarthritis had a prevalence ranging from 3.14% to 5.86% (See Table 1). Individuals in the five cohorts above the BMI under-19 level were all at significantly increased odds of developing glenohumeral osteoarthritis when compared to the BMI under-19 cohort (OR range: 1.21 – 1.92). Those in the three cohorts above BMI 30 were also at significantly increased odds of undergoing arthroplasty (OR range: 1.19 – 1.52).

Conclusion: Patients with higher BMI are at increased odds of osteoarthritis in the non-weight-bearing glenohumeral joint, despite the conventional belief that such an association is limited to hip and knee joints. This finding is in line with literature suggesting that adipokines may significantly contribute to cartilage degeneration, regardless of the forces exerted on the joint by bodyweight alone. The increased odds of undergoing arthroplasty in the higher BMI cohorts warrants further investigation but may represent the clinical manifestation of the basic science research demonstrating upregulation of neuropathic pain pathways in the presence of adipokines. As such, this finding may provide a rationale for studying anti-neuropathic pain regimens in higher BMI patients, with the ultimate potential benefit being the opportunity to prolong the time, or considerably reduce the need, to perform arthroplasty in this segment of the population that experiences glenohumeral osteoarthritis at an increased rate.

Table 1: Glenohumeral Osteoarthritis and Arthroplasty Rates and Odds Ratios by BMI Cohort

<table>
<thead>
<tr>
<th>BMI</th>
<th>&lt;19</th>
<th>19-24</th>
<th>25-29</th>
<th>30-34</th>
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<th>&gt;39</th>
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<td>N</td>
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<td>99479</td>
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<tr>
<td>GH OA (%)</td>
<td>3119 (3.14)</td>
<td>3740 (3.76)</td>
<td>4380 (4.40)</td>
<td>5082 (5.11)</td>
<td>5648 (5.68)</td>
<td>5834 (5.86)</td>
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<tr>
<td>Odds Ratio (95% CI)</td>
<td>REF</td>
<td>1.21*</td>
<td>1.42*</td>
<td>1.66*</td>
<td>1.86*</td>
<td>1.92*</td>
</tr>
<tr>
<td>GH Arthro. (%)</td>
<td>219 (7.02)</td>
<td>256 (6.84)</td>
<td>329 (7.51)</td>
<td>418 (8.23)</td>
<td>590 (10.45)</td>
<td>600 (10.28)</td>
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<tr>
<td>Odds Ratio (95% CI)</td>
<td>REF</td>
<td>0.97</td>
<td>1.08</td>
<td>1.19*</td>
<td>1.54*</td>
<td>1.52*</td>
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</table>

BMI= Body Mass Index; N= number of patients; GH OA= number of patients in cohort with glenohumeral osteoarthritis (% of cohort with GH OA); 95% CI= 95% confidence interval of odds ratio; GH Arthro. = number of patients who underwent glenohumeral arthroplasty (% of patients with GH OA in the cohort who also underwent arthroplasty); REF = reference cohort for odds ratio calculations; * indicates significance at α=0.05
Predictors of Major Bleeding and Major Adverse Cardiovascular Events in Patients with Peripheral Artery Disease: Insights from the EUCLID Trial

Rachael Ward; Zhen Huang; Frank W. Rockhold; Iris Baumgartner; Jeffrey S. Berger; Juuso Blomster; F. Gerry Fowkes; Brian Katona; Kenneth W. Mahaffey; Lars Norgren; Rajendra Mehta; Thomas J. Povsic; Sreekanth Vemulapalli; William R. Hiatt; Manesh R. Patel; W. Schuyler Jones

Background: Rates and predictors of major bleeding events in patients with peripheral artery disease (PAD) treated with antiplatelet medications are not known. This post-hoc analysis of the EUCLID trial aimed (1) to determine the incidence of minor/major bleeding in all patients, (2) to determine the predictors of major bleeding, and (3) to assess the impact of TIMI major bleeding on major adverse cardiovascular events (MACE).

Methods: EUCLID was a multicenter, randomized controlled trial of 13,885 symptomatic patients with PAD that tested the efficacy and safety of ticagrelor vs. clopidogrel for the prevention of major adverse cardiovascular events (MACE). The primary safety endpoint was TIMI major bleeding. Baseline characteristics were used to develop a multivariate model for TIMI major bleeding. The relationship between TIMI major bleeding and MACE was assessed by treating bleeding as a time dependent covariate, adjusting for other baseline covariates. The effects of the types of bleeding (procedural vs. non-procedural) on MACE was also assessed in a regression model.

Results: TIMI major bleeding was infrequent in EUCLID (0.94 per 100 patient years). TIMI major bleeding was similar for patients randomized to ticagrelor and clopidogrel (hazard ratio (HR) 1.10; 95% CI 0.84 - 1.43; p=0.49). After multivariable analysis of baseline characteristics, factors associated with TIMI major bleeding included advanced age, geographic region, Rutherford classification, and treatment with beta-blockers. The risk of a TIMI major bleeding was increased in both the procedure related (HR 2.85; 95% CI 1.73 – 4.70; p<0.0001) and non-procedure related (HR 5.09; 95% CI 3.71 – 6.97; p<0.0001) bleeding groups. The short-term effect of major bleeding on MACE was significant in both the procedure related (HR 5.9; 95% CI=1.73 – 3.71; p<0.0001) and non-procedure related (HR 44.24; 95% CI 30.13 – 64.93; p<0.0001) bleeding groups.

Conclusions: In patients with symptomatic PAD, older age, residing in North America, disease severity, and beta-blocker usage are all factors significantly associated with TIMI major bleeding. Patients who had a major bleeding event were significantly more likely to experience MACE. The relationship between major adverse cardiovascular events and major bleeding events should also be further explored for the development of a possible predictive risk score for MACE with bleeding.
Social Incentives and Patient-Reported Outcome Measure Survey Response Rate in the Orthopaedic Population: A Randomized Control Trial

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American Orthopaedic Society for Sports Medicine Sandy Kirkley Clinical Outcomes Research Grant

Background: Patient-Reported Outcome Measures (PROMs) represent an important tool for quality measurement and care improvement, but response to PROM surveys remains low. Social incentives, such as an offer of a charitable donation, have not been effective in increasing response rates for related surveys, but previous incentive offers lacked meaningful relevance to the patient populations under study. We tested the hypothesis that personalized social incentives would improve response rate to PROM surveys in a postoperative orthopaedic population.

Methods: Patients 18 years or older who were one to two years postoperative from an orthopaedic procedure and had an email address on file were randomized to one of four groups: 1) Control: no incentive offered, 2) Patient Donation: offer of a $5 donation to provide medical supplies to a pediatric orthopaedic patient, 3) Research Donation: offer of a $5 donation to a procedure-specific research program, 4) Explanation: detailed explanation of how survey response supports efforts to improve care quality. Patients were sent an email invitation with a link to a PROM survey. The primary outcome was the proportion of patients responding in each experimental group.

Results: 2901 patients were analyzed, with an overall response rate of 45.6%. Response rate was highest among patients in the Research Donation group (48.7%), followed by the Patient Donation group (45.4%), the Control group (44.5%), and the Explanation group (43.7%), though these differences were not statistically significant (p=0.239). In subgroup analyses, there was a significant difference in response by experimental group in males (Research Donation: 49.1%, Patient Donation: 44.5%, Control, 40.0%, Explanation: 39.1%, p=0.041) and patients younger than 58 (Research Donation: 39.9%, Control: 35.0%, Patient Donation: 31.7%, Explanation: 27.4%, p=0.004).

Conclusion: We found that personalized social incentives were not effective in improving overall PROM survey response rate in a postoperative orthopaedic population; however, they did affect response among younger and male patients. Therefore, social incentives could hold potential as a targeted strategy for increasing survey response rates in specific low-response groups, though additional cost-effective strategies will be necessary to reach response thresholds that enable health care stakeholders to practically and effectively utilize PROMs.
Diving in the clouds: Using modified decompression tables to mitigate the increased risk of decompression sickness in high altitude diving

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Funded in part by the US Navy

Background: Decompression sickness (DCS) is a serious and potentially fatal consequence of transitioning from a high pressure to a low pressure environment, as in the ascent from depth during a SCUBA dive. Inert gases, largely nitrogen, diffuse into the blood and the body tissues at high ambient pressure in a process known as “on-gassing.” As atmospheric pressure decreases during ascent, the solubility of these gases in tissue is reduced, and they form bubbles that are “off-gassed” through the pulmonary system. If the ascent is too rapid for the depth or duration of a dive, bubble formation occurs at a rate that cannot be matched by off-gassing capacity. These bubbles can expand and cause clots, obstruct vasculature, and directly impinge on tissue, resulting in headache, joint pain, paralysis, and death. The US Navy and other diving organizations publish decompression tables to articulate safe decompression protocols for different depths and durations of dives. Since atmospheric pressure at altitude is lower than at sea level, a diver at altitude experiences a greater pressure differential for the same given dive depth, and therefore a greater risk of DCS. To account for this difference, modifications have been proposed for altitude diving. The Cross Correction technique uses the ratio of pressure at altitude to calculate a “virtual depth,” which is then used in published decompression tables. However, these tables have not been experimentally tested. Diving at altitude is further complicated by the fact that rapid travel to altitude, such as flying from sea level to a city at high elevation, can commonly cause Acute Mountain Sickness (AMS). The symptoms of AMS, including headache, confusion, nausea, and numbness, not only directly impact the execution of the dive but overlap with symptoms of DCS and complicate its diagnosis.

Methods: 74 healthy human participants aged 18-40 years will be divided among five altitude/dive profiles. After remaining at atmospheric pressure equal to altitudes of 8000, 10000, and 12000ft for 12-48 hours to alleviate any AMS symptoms, participants will dive to depths of 60 and 100ft breathing either air or an enriched oxygen mix. Subjects are monitored for signs and symptoms of DCS for 12 hours post-dive, including cardiac imaging to document venous bubbles.

Results: 8 subjects have completed dives from to 60fsw for 30 minutes after acclimatizing to an altitude of 10,000ft. No subjects have shown any signs or symptoms of decompression sickness or venous gas emboli. One subject has had clinically diagnosed acute mountain sickness, and all scored above zero on the Lake Louise AMS scale at some point during the study.

Conclusions: This is experiment could verify the safety of Cross Corrections to decompression tables for diving at altitude. Data from these profiles will also be used to add to existing models of DCS. These will enable a better understanding of the physiology of decompression sickness and the effects of common variables on its development.
Intracerebral Hemorrhage in Patients with Brain Metastases Receiving Therapeutic Anticoagulation

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**Background:** Venous thromboembolism (VTE) is common in patients with solid malignancies, including those with brain metastases. Whether to anticoagulate such patients is debatable given the potential for intracerebral hemorrhage (ICH) in patients with brain metastases. We sought to evaluate the potential added risk of ICH in patients with brain metastases who receive therapeutic anticoagulation.

**Methods:** We performed a matched retrospective cohort study of 291 patients (100 who received therapeutic anticoagulation and 191 controls) with brain metastases managed at Brigham and Women’s Hospital / Dana-Farber Cancer Institute between 1998-2015. The cohorts were matched on age, Charlson comorbidity score, primary tumor type, and initial intracranial oncologic treatment. For each patient, all magnetic resonance imaging studies of the brain were reviewed to identify ICH. We also assessed whether intracerebral bleeds were extralesional (i.e., originating from and extending beyond the metastatic lesion and into the brain parenchyma) or resulted in neurologic symptomatology or death. Multivariable Cox regression was used for all time-to-event outcomes.

**Results:** The risk of ICH was comparable in patients receiving anticoagulation versus controls in the pre-anticoagulation period. However, after anticoagulation was initiated, we observed a trend to a higher rate of ICH (HR 1.31, 95% CI 0.95-1.80, p = 0.10) relative to controls, and the risk of extralesional ICH was significantly greater in patients receiving anticoagulation (HR 5.8, 95% CI 1.56-21.7, p = 0.009). Three patients in the anticoagulation cohort died of ICH compared to one patient in the control cohort (HR 5.68, 95% CI 0.59-54.8, p = 0.13). Receipt of anticoagulation was associated with a differentially higher risk of symptomatic ICH in patients with melanoma (HR 6.46, 95% CI 1.81-23.1, p = 0.004) versus other primary malignancies (HR 1.36, 95% CI 0.63-2.92, p = 0.44), p-interaction = 0.04. Receipt of anticoagulation was also associated with a differentially higher bleeding risk in patients who had experienced any ICH prior to anticoagulation initiation (HR 2.20, 95% CI 1.46-3.31, p <0.001) than in patients who did not (HR 0.68, 95% CI 0.40-1.15, p = 0.15), p-interaction <0.001.

**Conclusions:** Anticoagulation is associated with clinically significant ICH in patients with brain metastases, especially those with melanoma or prior ICH. The strength of the indication for anticoagulation and the risk of intracerebral bleeding on an individual patient level should be taken into consideration when deciding whether to initiate anticoagulation in patients with brain metastases.
Retinal Microvasculature and Neurodegenerative Changes in Alzheimer’s Disease and Mild Cognitive Impairment Compared to Community Controls

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Duke University Unrestricted Small Grant from Research to Prevent Blindness, Karen L. Wrenn Alzheimer’s Travel Award

Background: Current diagnosis of Alzheimer’s disease (AD) and mild cognitive impairment (MCI) is expensive, invasive, and not widely available. Cerebral microvascular changes in AD and MCI are inaccessible to existing in vivo imaging technologies. In contrast, the retinal vascular network can be directly visualized through imaging and provides a unique “window” to study cerebral microvascular pathology. Optical coherence tomography angiography (OCTA) is fast, non-invasive and inexpensive. We hypothesize that retinal microvasculature alterations and neurodegenerative changes in AD and MCI can be detected using OCTA and may serve as ocular biomarkers to aid in earlier diagnosis.

Methods: In this cross-sectional study (Clinicaltrials.gov NCT03233646), subjects with AD or MCI aged ≥50 years, along with community controls, were imaged using OCTA (Cirrus 5000, Carl Zeiss, Dublin, CA) at the Duke Memory Disorders Clinic. Exclusion criteria included non-AD associated dementia, diabetes, uncontrolled hypertension, demyelinating disorders, glaucoma, macular degeneration, or corrected visual acuity worse than 20/40. All subjects underwent Mini Mental State Examination (MMSE) to evaluate cognitive function. Both eyes were analyzed for vessel density (VD), perfusion density (PD), and foveal avascular zone (FAZ) area in the superficial capillary plexus (SCP, 3x3 mm scan and 6x6 mm scan, using an ETDRS grid overlay), ganglion cell layer (GCL) thickness, central macular thickness (CMT), and retinal nerve fiber layer (RNFL) thickness. Analysis was automated using AngioPlex software (Carl Zeiss). Multivariate statistical analysis was completed in STATA 12.1.

Results: 45 AD subjects (78 eyes), 41 MCI subjects (79), and 140 normal control subjects (264 eyes) were imaged. AD subjects had significantly decreased macular VD and PD in the 3x3 mm full and inner SCP regions (p<0.005) and 6x6 full SCP region (p<0.05) compared to control and MCI subjects. AD subjects had significantly decreased average GCL thickness (p<0.05) compared to control and MCI subjects. FAZ area, CMT and average RNFL thickness were not significantly different among groups.

Conclusions: AD subjects had significantly reduced macular VD and PD compared to both MCI and control subjects. Changes in the retinal microvasculature and GCL may mirror small vessel cerebrovascular and neurodegenerative changes in AD. These parameters may serve as surrogate non-invasive biomarkers in diagnosing AD and monitoring progression of MCI to AD.
The Natural History and Genetics of Drusenoid Pigment Epithelial Detachment Associated with Age-Related Macular Degeneration

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NIH Medical Research Scholars Program Fellowship

Background: Age-related macular degeneration (AMD) is the most common cause of vision loss in elderly individuals in industrialized countries. The hallmark of AMD is macular drusen. Drusenoid pigment epithelial detachment (DPED) is a related fundus lesion formed by the confluence of many drusen and measuring ≥ 350 µm in diameter. DPED has been shown to be an independent risk factor for progression to late AMD in the Age-Related Eye Disease Study (AREDS). While the genetic associations of AMD have been extensively studied, those of DPED have not. The purpose of this study was to investigate the natural history and genetics of DPED associated with AMD in the Age-Related Eye Disease Study 2 (AREDS2).

Methods: AREDS2 (2006 – 2012) was a multicenter, randomized, controlled clinical trial designed to study the safety and efficacy of adding lutein, zeaxanthin, and omega-3 fatty acids to the AREDS supplements on slowing progression to late AMD. AREDS2 eyes with DPED without late AMD at DPED detection were included in the natural history analyses. Baseline and annual stereoscopic fundus photographs were graded according to a standardized protocol to detect DPED and to evaluate for progression to late AMD and loss of ≥ three lines of visual acuity (VA). Genetic analyses included white AREDS2 participants with available genetic data and DPED without late AMD at DPED detection as well as a similar cohort from AREDS. We investigated five single nucleotide polymorphisms (CFH [rs10611670], C3 [rs2230199], CFI [rs10033900], C2/CFB [rs114254831], ARMS2 [rs10490924]) and genetic risk score (GRS) group for association with DPED development. Kaplan-Meier estimates and age- and sex-adjusted proportional hazards models were performed.

Results: Of the 4203 AREDS2 participants, 391 eyes of 325 participants were included in the natural history analyses. Mean (SD) follow-up time from DPED detection was 4.7 (0.9) years. Presence of DPED was associated with increased risk of progression to late AMD (hazard ratio [HR]=2.38, 95% confidence interval [CI]=1.99-2.84, p<0.001); 67% of eyes progressed to late AMD five years after DPED detection. DPED was associated with increased risk of ≥ three lines of VA loss (HR=3.08, CI=2.41-3.93, p<0.001); 46% of all eyes, with or without progression to late AMD, experienced vision loss at five years. Genetic analyses included 120 AREDS2 and 145 AREDS participants. Increasing GRS group (4 vs. 1) (HR=5.21, CI=2.55-10.66, p<0.001) was significantly associated with development of DPED. ARMS2 and C3 risk alleles reached nominal significance for association with DPED development but were not significant after Bonferroni correction.

Conclusions: This study replicates the results of previous natural history studies of eyes with DPED including the high rates of progression to late AMD and vision loss (regardless of progression to late AMD). The genetic associations may reflect genes associated with AMD progression.
Visual Acuity Assessment and Vision Screening Using a Novel Smartphone Application

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**Background:** While visual acuity (VA) is the best individual measure of ocular health, it can be difficult to assess in children. A novel smartphone-based application (Peek Acuity), validated in adults, uses a standardized algorithm to assess VA. Our objectives were to compare VA assessment between Peek Acuity and the pediatric ophthalmology clinical examination and evaluate Peek Acuity as a screening tool for referable ocular conditions (e.g. decreased vision, strabismus, nystagmus).

**Methods:** We prospectively recruited children aged 3-17 years presenting to a pediatric ophthalmology clinic who could follow instructions. Peek Acuity and standard clinic VA were assessed in randomized order. We compared VA assessments between the methods using intraclass correlation coefficient (ICC) and evaluated Peek Acuity’s ability to identify children with referable ocular conditions.

**Results:** ICC comparing VA assessed by Peek Acuity and standard clinical methods was 0.88 (95% confidence interval (CI): 0.83-0.92) for first and 0.85 (95%CI: 0.78-0.89) for second eyes examined. ICC among 3-5 year olds (preschool-aged children) was 0.88 (95%CI: 0.77-0.94) for first and 0.45 (95%CI: 0.13-0.68) for second eyes examined. Peek Acuity had a sensitivity of 81% for decreased vision and 69% for referable ocular disease. Sensitivity was highest among 3-5 year olds with decreased vision, 91%.

**Conclusions:** Overall, Peek Acuity VA assessment showed good correlation with that assessed by standard clinical examination methods, though preschool-aged children appeared more susceptible to examination fatigue. Peek Acuity performed adequately as a screening tool and appeared to have the greatest sensitivity among those with decreased vision and preschool-aged children.
**Tumor Microenvironment in Breast Cancer**

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_NIH ES024015, CA208852, CA216876, Eugene A. Stead Research Scholarship_

**Background**: The tumor microenvironment can influence the progression of breast cancer. Targeting active pathways within the tumor microenvironment may be especially beneficial for triple negative breast cancer (TNBC). Our study focuses on the phenotype of human adipose-derived stem cells (ASCs) with the BRCA1 mutation and the resulting effects on breast cancer cells. We hypothesize that the mutation of BRCA1, a DNA damage response protein, induces cell senescence in ASCs and promotes the secretion of inflammatory cytokines, which leads to increased tumor invasion and metastasis.

**Methods**: CRISPR/Cas9 was used to generate _de novo_ BRCA1-knockdown in human ASCs (BRCA1-KD). We have previously generated a human breast ASC cell line repository from mastectomy specimens. Breast ASCs from patients who have BRCA1 mutation (BRCA1-/-) and normal BRCA1 expression (WT) were also evaluated. Tumor proliferation was examined by co-culturing ASCs and luciferase-labeled TNBC cell line MDA-MB-231 in 5% FBS media over 8 days. Tumor invasion was evaluated using transwell assays, in which the CRISPR/Cas9 control and BRCA1-KD ASCs served as feeders. To investigate DNA damage in ASCs, immunofluorescence staining was performed for phosphorylated ATM, an established marker for DNA double strand breaks. The level of cell senescence was tested using beta-galactosidase assays. Finally, conditioned media was obtained from ASC lines after 48hr incubation and the inflammatory cytokine levels were evaluated using quantitative ELISA.

**Results**: We demonstrated 79.1% knockdown expression of the BRCA1 gene in our CRISPR/Cas9- mediated knockdown and confirmed decreased BRCA1 expressions in the two patients with BRCA1 mutation, respectively. Tumor growth _in vitro_ was significantly faster when co-cultured with BRCA1-KD ASCs (7.43% vs 5.16% increase at day4 comparing to day0, _P_=0.0074; 10.17% vs 6.57% at day6, _P_=0.036; 13.39% vs 8.02% at day8, _P_=0.045). Serving as feeders, BRCA1-KD ASCs induced significantly higher level of tumor cell migration (151±17.39 vs 66±13.17 cells migrated. _P_=0.0171). Immunofluorescence staining revealed higher level of phosphorylated ATM activation in BRCA1-KD cells compared to controls (62.73±3.12 vs 45.4±2.91%. _P_=0.0155). The beta-galactosidase assay demonstrated more cell senescence in both BRCA1-KD and patients with BRCA1 mutation (7.9±0.25% vs 0.17±0.17%, _P_<0.0001 in BRCA1-KD; 41.7±2.30% vs 18.4±2.34%, _P_<0.0001 in patient samples). By performing ELISA assay on supernatant of BRCA1-KD and control ASCs, we found significantly higher level of IL-8 inflammatory cytokine (2.57±0.32-fold change, _P_=0.0049).

**Conclusions**: BRCA1 mutation in adipose-derived stem cells leads to increased inflammatory cytokine production via a DNA damage-mediated cell senescence pathway. The effect from ASCs increases tumor proliferation and invasion. This interaction between ASCs and breast cancer cells can be targeted for more effective anticancer therapies in the setting of high-risk breast cancer.
Analysis of Cost and Attribution of Newly ESRD Patients in Duke Connected Care

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Eugene A. Stead Student Research Scholarship

**Background:** The cost of managing the transition from chronic kidney disease (CKD) to end-stage renal disease (ESRD) is a major financial burden for the healthcare system. The Medicare accountable care organizations (ACOs) provide coordinated quality care for patients to create savings. However, little is known about the participation of ESRD patients. Our objective is to identify characteristics of newly ESRD patients within Duke’s ACO (Duke Connected Care).

**Methods:** Centers for Medicare and Medicaid Services (CMS) provides Duke Connected Care with information regarding patient entitlement status, PCP, and associated quarterly costs. Using this data set, patients deemed newly ESRD in 2016 Q2 through 2016 Q4 were extracted and further identified as being “previously attributed” to Medicare and “newly attributed”. Statistical analysis was used to determine average quarterly cost of care and cost percent change compared to preceding quarters. MaestroCare was additionally used to abstract patient demographic information, associated comorbidities, dialysis start date, initial vascular access type and location, and nephrology specialty usage.

**Results:** A total of 271 patients were identified by the CMS record as being newly ESRD in 2016 Q2 through 2016 Q4. MaestroCare chart review of dialysis start dates refined this number to 47 patients, with 35 being previously attributed to Medicare and 12 being newly attributed. In general, the newly attributed are younger (55.6 years vs 69.3 years), more likely to be African American (67% vs 46%), more likely to have no primary care provider (50% vs 11%), and more likely to have no predialysis nephrology care (67% vs 31%). Interestingly, previously attributed patients were more likely to begin dialysis in the hospital setting (89% vs 67%); furthermore, while the previously attributed tended to have an early AV fistula placement (24% vs 17%), both groups used had the same rate of initial AV fistula usage (17%). Cost-wise, the average first quarter cost was $42,961 vs $33,089 (newly vs previously attributed).

**Conclusions:** This retrospective study is the first to compare the cost and characteristics of newly attributed and previously attributed ESRD patients within the Medicare ACO model. Since becoming ESRD automatically qualifies all patients for Medicare, we have the unique opportunity of using this date of transition as a common starting line for comparison. On first impressions, we can see that newly attributed patients are much less connected to the medical system, both in terms of primary care usage and nephrology specialist usage, which in turn may explain the significantly younger age of this group. Furthermore, we see that the vast majority of patients in both groups begin dialysis in the hospital and require temporary venous access, options which greatly increase the cost of care. The low percentage of previously attributed patients who have early AV fistula creations (24%) suggests that nephrology specialists may need to be more rigorous in anticipating the ESRD transition. Ultimately, the cost of care for the ESRD transition represents a toxic financial burden for both the medical system and patients, and more research is needed to further understand this.
Chemotherapy Potentiates Immunotherapeutic Efficacy of Recombinant Oncolytic Poliovirus for Treatment of Glioblastoma

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*HHMI Medical Research Fellowship*

**Background:** Glioblastoma (GBM), the most common primary malignant brain tumor in adults, is a highly lethal cancer with nearly all patients experiencing tumor recurrence after standard of care surgery, radiation, and chemotherapy. Thus, novel therapeutic strategies are urgently mandated. PVSIPO is a highly-attenuated, recombinant polio:rhinovirus chimera that has demonstrated promising and robust clinical and radiographic responses in phase I clinical trials for recurrent GBM. An unexpected discovery during the phase I trial was the achievement of complete and durable responses in patients who were treated with single-dose chemotherapy months after tumor progression following PVSIPO infusion. This led to the initiation of an ongoing phase II trial comparing the efficacy of PVSIPO and single-dose chemotherapy to PVSIPO alone. The goal of this study is to elucidate the therapeutic mechanisms of combined PVSIPO and single-dose lymphodepletive chemotherapy using syngeneic mouse tumor models. We hypothesize that post-PVSIPO lymphodepletive chemotherapy elicits an ‘immunologic reset’ that unmasks antitumor immune responses initially generated by PVSIPO.

**Methods:** CD155-transgenic C57Bl/6 mice were implanted with $1 \times 10^6$ CT2A murine GBM or TRAMPC2 prostate cancer cells subcutaneously on the right flank. Mice received intratumoral injections of serum-free DMEM (control) or mRIPO (mouse-adapted PVSIPO, $5 \times 10^6$ plaque forming units); the following week, they were treated with intraperitoneal DMSO (control) or temozolomide (TMZ, 400 mg/kg). Tumor caliper measurements were performed twice a week to assess tumor progression until the survival endpoint was reached (tumor volume $\geq 1000 \ mm^3$). Blood and tumor samples were harvested from each group for flow cytometric analyses of peripheral and tumor-infiltrating immune cells.

**Results:** Combination treatment with mRIPO and TMZ resulted in decreased tumor growth and increased overall survival compared to mRIPO alone (CT2A) and either treatment alone (TRAMPC2). Early flow cytometry data suggest that the addition of lymphodepletive chemotherapy may induce CD8+ effector T cell infiltration of the tumor and increase the intratumoral CD8+/regulatory T cell ratio.

**Conclusions:** This study demonstrates that combined mRIPO and single-dose lymphodepletive chemotherapy confers survival benefit and delayed tumor growth in syngeneic mouse models of GBM and prostate cancer. Flow cytometric analyses of tumor samples reveal a shift towards the effector CD8 T cell phenotype and an increase in the CD8+/regulatory T cell ratio, suggesting that chemotherapy may synergize with PVSIPO to restore antitumor immunity within the tumor microenvironment. Future experiments will utilize flow cytometry at various time points after chemotherapy administration to further dissect the therapeutic mechanism of the combination treatment.
Limitations of Available Blood Products for Massive Transfusion during Mass Casualty Events at US Level 1 Trauma Centers

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Background: Exsanguination remains a leading cause of preventable death in traumatically injured patients. To better treat hemorrhagic shock, hospitals have adopted massive transfusion protocols (MTPs) which accelerate the delivery of blood products to patients. There has been an increase in mass casualty events (MCE) worldwide over the past two decades. These events can overwhelm a responding hospital’s supply of blood products. This study investigated the ability of US trauma centers (TCs) to meet the blood product requirements of MCEs with a computerized model.

Methods: Cross-sectional survey data of on-hand blood products was collected from 16 US level-1 TCs. A discrete event simulation model of a TC was developed based on historic data of blood product consumption during MCEs. Each hospital’s blood bank was evaluated across increasingly more demanding MCEs using modern MTPs to guide resuscitation efforts in massive transfusion (MT) patients. TCs were evaluated on their ability to meet the blood product requirements of patients using a MTP for MT patients, the maximum number of patients treated, and which blood products they exhausted.

Results: A total of 9,000 simulations were performed on each TC’s data. Under the least demanding MCE scenario, the median size MCE in which TCs failed to adequately meet the blood product requirements of patients was 80 patients (IQR 60-120), not considering platelets, and 50 patients (IQR 20-90), considering platelets. More than half of surveyed TCs are unable to adequately treat four MT patients, not considering platelets, and two MT patients when considering platelets. Five TCs exhausted their supply of O- red blood cells (RBCs), six exhausted their AB plasma supply, and five had a mixed exhaustion picture. Platelet supplies were exhausted prior to RBCs or plasma supplies in 10 TCs.

Conclusion: Assuming a TC’s ability to treat patients is limited only by their supply of blood products, US level-1 TCs lack the on-hand blood products required to adequately treat patients following a MCE. Use of non-traditional blood products, that have a longer shelf life, may allow TCs to better meet the blood requirement needs of patients following larger MCEs. By identifying limitations in their blood product supplies, TCs are able to make changes to their MTPs allowing their supplies to be optimized for transfusion.