



NIH Medical Research Scholars Program

2014-2015 Scholars & Abstracts



About the Medical Research Scholars Program

This publication lists the 2014-2015 Scholars of the National Institutes of Health's (NIH) Medical Research Scholars Program (MRSP), and outlines their research studies in their year-long participation in the program.

MRSP builds on decades of experience at NIH in training clinician-scientists, and provides outstanding U.S. medical, dental and veterinary students with advanced training in laboratory, clinical and translational research. Its one-year intensive training program enables the most promising clinicians to understand the biological underpinnings of disease and translate basic science into health care interventions.

Launched by NIH in 2012, MRSP combines and re-envisioned two highly successful NIH training initiatives: the Clinical Research Training Program (CRTP) that operated from 1997 to 2012 and the HHMI-NIH Research Scholars Program that operated from 1985 to 2012. The program is designed for students who have completed their initial clinical rotations and are primarily between their third and fourth years of professional school. In the course of their year at NIH, MRSP Scholars work with a Tutor who provides research support and career guidance, and a Mentor, who helps them to develop a year-long laboratory, clinical or translational research project that aligns with their clinical interests and career goals. Based on the nature of their project, they conduct their research at one of the 27 Institutes or Centers within the NIH intramural program.

MRSP is distinguished from other training programs by the Scholars' unique access to the full range of NIH resources. These include laboratories and clinical research facilities that are among the most extensive and highly regarded in the world; access to the NIH's 27 intramural Institutes and Centers; NIH lectures and tutorials on seminal research and new clinical discoveries; and teaching rounds at the NIH Clinical Center, America's Research Hospital. Scholars spend the majority of their time on their research but they participate in a complementary program of professional development, enrichment, scholarship and leadership opportunities.

Recognizing that successful biomedical research depends on the talent and dedication of the scientific workforce, NIH supports innovative training programs like MRSP that foster scientific creativity and exploration. NIH's goal is to strengthen our nation's research capacity, broaden our research base and inspire a passion for science in current and future generations of researchers.

For more information about MRSP or to learn about opportunities to support the program, please contact the Development Office at Development@fnih.org.

2014-2015 Medical Research Scholars Program

Scholar: Steven Abboud
School: Saint Louis University School of Medicine
Mentor: W. Marston Linehan, M.D., Chief, Urologic Oncology Branch
Institute: National Cancer Institute (NCI)
Research: Kidney and Prostate Cancer



Renal cell carcinoma (RCC) affects more than 270,000 individuals annually worldwide and in the United States, the incidence has grown at a rate of 1.4% per year over the past 10 years. RCC represents a collection of cancers we can differentiate by histology including papillary type I and II. Specific genetic alterations have been associated with specific RCC histologies and the predisposition to these cancers can be genetically inherited.

The hereditary form of type II papillary RCC, called hereditary leiomyomatosis and renal cell carcinoma (HLRCC), is an autosomal dominant hereditary cancer syndrome in which patients are at risk for development of cutaneous leiomyomas, uterine leiomyomas and RCC. HLRCC kidney tumors

are very aggressive and there are currently no effective treatments available. HLRCC is characterized by a germline mutation of the gene for the Krebs cycle enzyme fumarate hydratase (FH), which converts fumarate to malate.

In normal cells, the prolyl hydroxylase domain (PHD) enzymes hydroxylate the HIF transcription factors to allow for degradation. In HLRCC, fumarate accumulates and inhibits PHD, resulting in accumulation of HIF1 and the subsequent increased expression of downstream target genes such as VEGF and TGF α , which are critical for increased vasculature and aerobic glycolysis. We sought to target these gene products to inhibit the vasculature and metabolism of these tumors, in theory inhibiting proliferation and metastasis. We combined bevacizumab, a monoclonal VEGF antibody to target the vasculature, and erlotinib, an EGFR tyrosine kinase inhibitor to target aerobic glycolysis, in a xenograft murine model using the human HLRCC cell line UOK262. Our data showed a trend for retarded tumor kinetics as well as increased survival of the group treated with both bevacizumab and erlotinib compared with the control and single agent therapies groups. Our findings provide the foundations for a promising novel therapeutic strategy for HLRCC patients and their families.

2014-2015 Medical Research Scholars Program

Scholar: Abdulkareem Agunbiade
School: University of Chicago, Pritzker School of Medicine
Mentor: Michael Ward, M.D., Senior Investigator, Clinical Trials and Outcomes Branch
Institute: National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)
Research: End Stage Renal Disease and Health Disparities



Hemodialysis has changed the outlook of end stage renal disease from a once fatal condition to a chronic disease; however, its use still carries significant morbidity and mortality. Currently, there are approximately 450,000 Americans on dialysis, yet even with recent advances in medical care, these individuals have a 40.4% adjusted five year survival rate. When stratifying survival rates on dialysis by ethnicity, many studies have demonstrated that blacks on dialysis have a survival advantage compared to whites. However, this is paradoxical to expectation, given the increased prevalence of hypertension, diabetes, and other co-morbidities among black subjects, since these co-morbidities correlate with a decreased survival rate on dialysis. We hypothesized that this paradoxical

survival advantage can be explained by the increased transplantation and withdrawal rates from dialysis in whites compared to blacks. Consideration of these two factors might account for the greater number of healthy white subjects who undergo transplantation as well as the greater number of sicker white subjects who withdraw from dialysis compared to blacks, therefore allowing a more accurate estimation of the true mortality rate on dialysis by ethnicity. We used a Cox regression model with competing risks in order to test if the survival advantage of blacks, and other ethnicities, relative to whites on dialysis persists. In our analysis, we used both renal transplantation and withdrawal from dialysis as the outcomes competing with death on dialysis. Also, we controlled for demographic and socioeconomic factors, in addition to various co-morbidities. With this model, we discovered that there was no significant survival advantage for blacks on dialysis compared to non-Hispanic whites. On the other hand, Hispanics had a significant survival advantage compared to non-Hispanic whites in all age groups except the oldest age group. These results suggest that the paradoxical survival advantage in blacks seen in the literature is misleading and most likely explained by social/access issues within these competing risks.

2014-2015 Medical Research Scholars Program

Scholar: Sara A. Armaiz Flores
School: University of Puerto Rico Medical Sciences Campus
Mentor: Jack A. Yanovski, M.D., Ph.D., Director, Section on Growth and Obesity
Institute: Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)
Research: Evaluating Weight Status and Sex as Moderators of the Association of Serum Leptin with Bone Mineral Density in Children and Adolescents



Both bone mineral density and serum concentrations of the hormone leptin are positively associated with children's body mass index (BMI). Animal studies suggest that leptin may adversely affect bone mineral density, but clinical studies in children and adults have yielded conflicting results. We investigated associations between leptin and bone parameters in a large pediatric cohort enriched for overweight and obese children and adolescents, and we examined if BMI z-scores (BMIz) and sex moderated these associations. A convenience sample of 830 healthy children (mean age=11.4±3.1 years; 75% female; 47% non-Hispanic white; BMIz=1.5±1.1) were studied.

Fasting leptin was measured with ELISA; body

composition was determined by dual-energy x-ray absorptiometry (DXA). Main effects for leptin and leptin's interactions with sex and BMIz were examined using hierarchical linear regressions for appendicular, pelvis, and lumbar spine bone area (BA), bone mineral content (BMC), and bone mineral density (BMD), controlling for demographic, pubertal development, and anthropometric variables. After adjusting for covariates, we found leptin was negatively and independently associated with lumbar spine BA and BMC, pelvis BA, and leg BA ($p < .05$). There were no significant interaction terms for BMIz and leptin. In boys, there were negative correlations between leptin and BMC at all bone sites examined ($p < 0.002$). Subtotal and lumbar spine BA ($p < 0.008$) and leg and arm BMD were also negatively correlated in boys. Conversely in girls, leptin was positively correlated with arm and leg BMD ($p < .05$). Independent of body size, leptin is negatively associated with BMD, BMC, and BA; this association is moderated by sex, not BMIz. Boys with higher leptin levels have an increased risk of acquiring lower BMC.

2014-2015 Medical Research Scholars Program

Scholar: Anna M. Brown
School: Duke University School of Medicine
Mentor: Peter L. Choyke, M.D., FACR, Director, Molecular Imaging Program
Institute: National Cancer Institute (NCI)
Research: Novel Imaging Analysis Techniques for Prostate Cancer



Prostate cancer is the second leading cause of cancer death in men. Recently, multi-parametric magnetic resonance imaging (mpMRI) and targeted biopsies have improved the ability to detect clinically significant prostate cancer, while reducing the diagnosis of low grade tumors. Nevertheless there is still a 5-10% rate of missed cancers. Thus, further optimization is needed to improve lesion detection and characterization, particularly in the setting of biochemical recurrence (BCR). A substantial number of patients present with clinically localized yet high-risk disease. They have the highest risk of BCR and eventual prostate cancer mortality, but efforts to risk-stratify patients based on pretreatment characteristics remain limited.

Computer-aided diagnosis (CAD) is one possible strategy for improving the detection of clinically significant cancers. It can be used to show areas of higher likelihood of clinically significant cancer. We evaluated the performance of a novel prostate CAD tool for lesion detection and reproducibility. Of the 54 consecutive patients and 307 lesions evaluated, we found that CAD was approximately equal to prostate mpMRI but CAD showed higher reproducibility. The combination of CAD plus mpMRI had the highest positive predictive value (PPV), which indicates promise for helping readers of different experience levels achieve similar PPVs >0.90 .

To better understand which features on pretreatment MRI correlate with risk of disease recurrence, we used texture analysis, which quantifies image intensity variation otherwise invisible to the naked eye. 18 patients with BCR following radical prostatectomy were matched to an 18 patient cohort without BCR on the basis of age, race, and preoperative prostate specific antigen. A model constructed from texture features distinguished the matched cohort with an area-under-the-curve of 0.87. This is superior to blood-based predictors of tumor aggressiveness. Further work remains to test this model prospectively, with the ultimate goal to identify patients who would benefit from early adjuvant therapies.

2014-2015 Medical Research Scholars Program

Scholar: Cortlyn Brown
School: Yale University School of Medicine
Mentor: Kathleen Merikangas, Ph.D., Chief, Genetic Epidemiology Research Branch
Institute: National Institute of Mental Health (NIMH)
Research: Specificity of Familial Aggregation and Cross-transmission of ADHD and Mood Disorder Subtypes in a Community-based Sample



Comorbidity between attention deficit hyperactivity disorder (ADHD) and mood disorders including both major depression (MDD) and bipolar disorder (BD) has been well established in both adults and adolescents (Kessler et al, 2005; Kalaydjian and Merikangas 2008; Larson, Russ et al. 2011). There is limited information on the familial patterns of comorbidity of mood disorder subtypes and ADHD in large family studies of the full range of mood disorders. The goal of this study is to investigate patterns of comorbidity and familial transmission of ADHD, bipolar, and major depression in a nonclinical sample of probands with a broad range of mood and comorbid conditions. The FAMILY STUDY is a large nonclinical nationally representative study

of probands with a broad range of mood and comorbid conditions and their relatives. The FAMILY STUDY methodology has been described elsewhere (Merikangas, Cui et al. 2014). Briefly, probands were recruited from a screening program in the greater Washington, DC metropolitan area. Direct semi-structured interviews, or structured family history from multiple informants, were used. Best estimate diagnostic procedure was performed on the NIMH Family Study Diagnostic Interview for Affective Spectrum Disorders. Statistical significance was determined by mixed effects non-linear regression models. ADHD models were controlled for proband and relative mood disorders, age, and sex. Mood disorder models were controlled for proband and relative ADHD, age, and sex. There is specificity of familial aggregation of mood disorder subtypes and ADHD. The lack of cross-aggregation of mood disorder subtypes and ADHD suggests that non-familial factors are likely in play. ADHD clinical severity decreased stepwise from comorbid MDD to BD2 to BD1. Clinical interventions and screening should, therefore, include comorbidity when assessing the ADHD patient.

2014-2015 Medical Research Scholars Program

Scholar: Harrison Cash
School: Case Western Reserve University School of Medicine
Mentors: Clint Allen, M.D., Clinical Collaborator, Otolaryngology Surgeon-Scientist Development Program, NIDCD; Carter Van Waes, M.D., Ph.D., Chief, Head and Neck Surgery Branch Clinical Director, NIDCD
Institute: National Institute on Deafness and Other Communication Disorders (NIDCD)
Research: mTOR and MEK1/2 Inhibition Modulate Tumorigenesis and the Immune Microenvironment in a Syngeneic Model of Oral Cavity Cancer



Head and neck squamous cell carcinomas (HNSCCs) are known to commonly harbor coactivating mutations in the PI3K/mTOR and MAPK pathways, which drives tumorigenesis. Cross talk between these two signaling networks likely accounts for the fact that inhibition of only one pathway arm with targeted therapies has not fulfilled expectations as a cancer treatment. In addition to their role in malignancy, PI3K/mTOR and MEK signaling is known to be integral for proper development of various myeloid cell subsets. Therefore, while combining targeted therapies to inhibit both PI3K/mTOR and MEK cascades may be beneficial for improving the efficacy in treating HNSCCs, it may also collaterally damage the host immune response and alter treatment outcomes. Herein, we

analyze the effects of the mTOR inhibitor, rapamycin, as well as the MEK1/2 inhibitor, PD901, either alone or combined in a syngeneic model of mouse HNSCC.

Utilizing both a poorly immunogenic and a potentially immunogenic mouse HNSCC cell line, tumors were transplanted into immunocompetent C57Bl/6 mice and allowed to reach an adequate volume before beginning a 21-day treatment regimen with control, rapamycin, PD901, or combination therapy. Following treatment, subsets of mice were sacrificed for tissue collection, and the remaining mice were saved for survival analysis. Interestingly, immunogenic tumors demonstrated a durable response after cessation of rapamycin that was not apparent with PD901. This effect could not be accounted for by superiority of rapamycin in eliciting intrinsically anti-tumor effects as determined by in vitro or in vivo experiments. However, flow cytometry of the tumors revealed marked suppression of a specific, CD8⁺ effector T-cell response in MEK inhibitor-treated versus rapamycin or control ($p < 0.05$), as measured by reduced tumor-associated antigen specific cells and CD8 activation marker CD107. Further experiments should characterize the potential immunologic sequelae of drugs so that they may be rationally combined with potential immunotherapies.

2014-2015 Medical Research Scholars Program

Scholar: Raju Chelluri
School: SUNY Upstate Medical University
Mentor: Leonard M. Neckers, Ph.D., Senior Investigator, Urologic Oncology Branch
Institute: National Cancer Institute (NCI)
Research: Heat Shock Protein 90 Inhibition Combined with Targeted Therapies Synergistically Effects Cytotoxicity in Bladder Cancer Models



In eukaryotes, heat shock protein 90 (HSP90) functions as a proteostasis center that modulates responses to environmental stress. Cancer cells can utilize the molecular chaperone's function to protect over-expressed or mutated oncoproteins—such target proteins are referred to as clients—from misfolding and degradation. Thus, HSP90 is recognized as a crucial facilitator of “oncogene addiction” and cancer cell survival. HSP90 inhibitors have been promising in vitro and in vivo as anti-tumor agents, especially when a highly HSP90-dependent client protein drives the tumor. Despite this, initial clinical trials of HSP90 inhibitors were unsuccessful due to a variety of reasons, such as poor patient selection and the observation that HSP90 inhibition

frequently leads to cytostasis rather than cytotoxicity. Novel combination strategies of HSP90 inhibitors with other agents are being actively sought, including therapies with synergistic effects. The purpose of my work was to investigate whether combination therapy, centered on HSP90 inhibition along with targeted cytotoxic or proteostatic agents, was superior to monotherapy in bladder cancer (BCa) models. We hypothesized that such combination therapy will synergize to increase cytotoxicity (CT) in BCa.

We found that BCa cell lines treated with human recombinant TRAIL, the HSP90 inhibitor 9090 or the proteasome inhibitor NPI0052 alone, demonstrated cytotoxicity. When TRAIL was given in combination with either 9090 or NPI0052, all of the BCa lines, except the normal urothelium, showed augmented or sustained suppression of cell growth. In a high-grade urothelial cell line, TRAIL and 9090 were both shown to be highly potent with IC50 values in the nano range. Combination index calculations demonstrated that synergy between 9090 and TRAIL inhibition of HSP90 caused degradation of the apoptotic inhibitor FLIP, in a similar pattern to known “client” proteins of HSP90. We conclude therefore that combination therapy centered on HSP90 is effective across a range of model BCa systems and is synergistic with other targeted agents.

2014-2015 Medical Research Scholars Program

Scholar: Anna Chichura
School: Georgetown University School of Medicine
Mentor: Nicholas P. Restifo, M.D., Senior Investigator, Surgery Branch
Institute: National Cancer Institute (NCI)
Research: Adoptive Cell Transfer Targeting the Programmed Death-1 Receptor in Melanoma



Melanoma is currently the fifth most common cancer in the United States; the 5-year survival rate for patients with stage IV metastatic melanoma treated with dacarbazine is about 10%, but advances in immunotherapy with monoclonal antibodies have increased the 5-year survival rate to about 20%. The immunotherapy with the greatest impact on survival is adoptive cell transfer (ACT). ACT is a type of cancer immunotherapy in which antitumor lymphocytes are identified *ex vivo*, expanded to large numbers, and re-infused into the same cancer patient. A major factor that limits the successful use of ACT in melanoma and other solid cancers is the identification of the diverse T cell receptor (TCR) repertoire that recognizes mutation-

specific antigens expressed on tumor cells but not on essential normal tissue. It has been demonstrated that programmed death receptor-1 (PD-1) expression on CD8+ tumor infiltrating lymphocytes (TILs) accurately identifies clonally expanded tumor-reactive cells. However, it is not known if sequencing TCRs from PD-1-expressing (PD-1+) TIL will identify mutation-reactive TCRs that can mediate tumor regression *in vivo*.

To test this hypothesis, we assessed the TCRs expressed by PD1+ T cells in tumor-bearing mice. We identified the sequences of paired TCR α and TCR β chains of CD3+ PD1+ TIL using a single cell sequencing based approach. TCR β deep sequencing revealed that the CD3+ tumor infiltrating T cell population was more oligoclonal than the CD3+ splenic T cell population, however there was no dominant V β clonotype that was shared among TIL from different mice. The receptors identified by TCR single cell sequencing will be retrovirally transduced into mouse T cells and adoptively transferred to tumor-bearing mice to determine if the TCRs derived from PD1+ TIL confer tumor specificity and have therapeutic potential in an ACT setting.

2014-2015 Medical Research Scholars Program

Scholar: Bartholt Clagett
School: University of Pennsylvania Perelman School of Medicine
Mentor: Philip Shaw, M.D., Ph.D., Stadtman Investigator, Neurobehavioral Clinical Research Section, Social and Behavioral Research Branch
Institute: National Human Genome Research Institute (NHGRI)
Research: Association Between Amygdala Volume and Emotion Dysregulation in Patients with Attention Deficit Hyperactivity Disorder



Emotion regulation refers to the ability to modulate emotional responses in order to engage in adaptive, goal directed behavior. Failures of such regulation, or emotion dysregulation (ED), result in temper outbursts and aggressive behaviors, and are a major contributor to childhood psychopathology. It is estimated that 11 percent of children in the United States have Attention Deficit Hyperactivity Disorder (ADHD) and that 24-50% of those children show some degree of ED. Prior research has shown abnormal amygdala activation in those with ED and ADHD when processing emotional stimuli. Based on these findings, we further hypothesized that: (1) morphological differences in the amygdala are associated with ED; (2) these associations

are constant across development. We examined a population of children and adults enriched for individuals with both ED and ADHD, including 301 children (age: 10.6 ± 2.9 ; 65% males, 113 ADHD, 188 typically developing) and 160 adults (age: 28.9 ± 10.1 , 56% males, 47 ADHD, 113 no history of psychiatric disorders). ED was assessed using the dysregulation profile of the Child Behavior Checklist, a parent report measure of behavioral problems, and the impulsivity/emotional lability scale of the Conners Adult ADHD Rating Scale, a self report measure of ADHD-related problems in adulthood. Amygdala volumes were defined from 3-Tesla and 1.5-Tesla magnetic resonance scans using segmentation software. Regression analysis of amygdala volumes against dysregulation scores, adjusting for intracranial volume, full scale IQ, diagnosis, scanner type (1.5T or 3T), and family membership were performed. Children showed a significant association between increased ED and decreased total amygdala volume ($b=-1.28$, $t=-2.32$, $p=.02$). Adults showed no association between ED and amygdala volume ($b=-0.35$, $t=-0.17$, $p=0.86$). These results provide further evidence that the amygdala plays a key role in ED in childhood, but contrary to our hypothesis, this association does not appear to persist across development into adulthood.

2014-2015 Medical Research Scholars Program

Scholar: Alex Dinh
School: University of Buffalo School of Medicine and Biomedical Sciences
Mentor: Jeffrey Kopp, M.D., Chief, Kidney Diseases Branch
Institute: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Research: Role of APOL1 in Intracellular Cholesterol Homeostasis



Apolipoprotein L1 (APOL1) circulates in human plasma bound to high-density lipoprotein (HDL). Individuals carrying two copies of APOL1 risk alleles, termed G1 and G2, are at increased risk of developing a form of chronic kidney disease called focal segmental glomerulosclerosis (FSGS) compared to individuals carrying the wild type (G0) allele. The G1 and G2 allelic variants are found primarily in individuals of Sub-Saharan African descent and are thought to have evolved as a form of innate immune adaptation against trypanosomal infection, which causes sleeping sickness. As the mechanism by which these variants cause kidney disease is unknown, our lab has worked on identifying pathways that are affected by APOL1 and how alterations in these pathways caused by the risk variants can increase susceptibility to kidney disease.

Given the association of circulating APOL1 with HDL and the importance of intracellular cholesterol in regulating the localization and function of proteins that are key to maintaining the filtration barrier of the kidney, we investigated the manner in which APOL1 affects intracellular cholesterol homeostasis. We found that activated THP-1 cells stably transfected to express APOL1 had increased cholesterol efflux into both nascent HDL particles (apolipoprotein A1) and into total HDL (PEG-treated plasma) compared to cells expressing a control vector. We then demonstrated that cells expressing APOL1 had increased resistance to amphotericin B lysis, a surrogate for plasma membrane cholesterol content and membrane fluidity. Using an Amplex Red Cholesterol Assay, we showed that APOL1 expressing cells did not have altered total cellular cholesterol, which suggests that the APOL1-mediated differences in both cholesterol efflux and amphotericin B lysis are due to differences in membrane fluidity. Future experiments will further characterize membrane fluidity using fluorescence anisotropy and quantify changes in plasma membrane protein composition using mass spectroscopy.

2014-2015 Medical Research Scholars Program

Scholar: Michele Fascelli
School: Jefferson Medical College of Thomas Jefferson University
Mentor: Peter A Pinto, M.D., Head, Prostate Cancer Division, Urologic Oncology Branch
Institute: National Cancer Institute (NCI)
Research: MRI/US Fusion-Guided Targeted Biopsy Detects Clinically Significant Prostate Cancer in the Central Gland



The utility of multiparametric MRI (mpMRI) has increasingly been adapted for use in anatomical and functional delineation of prostate cancer burden, with an increasing role in detection of disease. Zonal distributions of prostate cancer have historically been cited as occurring in 75% of cases in the peripheral zone (PZ) and in 25% of cases in the central gland (CG). Characterization of disease burden by zonal anatomy may alter treatment recommendations. Accurate identification of the index lesion, the lesion which drives pathologic progression, can impact oncological efficacy of whole-gland treatment.

We aimed to assess the performance of MRI/ultrasound fusion-guided biopsy for detecting and characterizing CG lesions. Retrospective review of 1003 patients revealed 2570 targeted lesions. Targets and patients were stratified by zone and cancer detections rates were tabulated by location. These were then correlated with PSA, Gleason score, prostate volume, PSA density, and MRI suspicion. Patients with CG lesions on mpMRI were older, had a significant history of prior biopsies, higher PSA values, larger prostate volumes, and, higher PSA densities. On multivariate analysis, age (OR: 1.03) and PSA (OR: 1.01) of the patients were associated with increased odds of CG lesions ($p < 0.05$). Targeted biopsy of mpMRI-identified lesions resulted in upgrading of 30% of PZ lesions. Comparatively, CG involvement was found to be associated with increased upgrading, with 60% of upgraded males being from their CG lesion ($p < 0.0001$). These CG index lesions accounted for nearly one-third of all cancer positive males. PSA (OR: 1.16) and prior biopsy history (OR: 6.91) were significant predictors of upgrading by a CG index lesion from Gleason 3+4 to Gleason 4+3 or higher disease ($p < 0.05$). Clinically, these results suggest that mpMRI may aid in identifying disease outside of the traditional biopsy template, and therefore the two modalities might serve in a complementary fashion.

2014-2015 Medical Research Scholars Program

Scholar: Michael J. Feldman
School: University of Rochester School of Medicine and Dentistry
Mentor: Prashant Chittiboina, M.D., Staff Clinician, Surgical Neurology Branch, NINDS
Institute: National Institute of Diabetes and Digestive and Kidney Diseases (NINDS)
Research: Protein Phosphatase 2A in Pediatric Brain Tumors

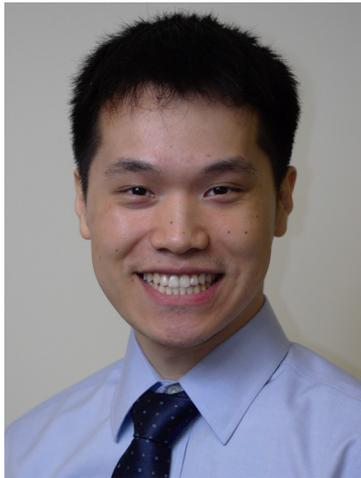


Medulloblastoma is the most common malignant brain tumor in children. Current treatment strategies combining surgical resection, chemotherapy and radiation are variably effective and often result in significant morbidity. Using the small-molecule Protein Phosphatase 2A (PP2A) inhibitor LB-100, we were able to induce sensitivity to cisplatin in the medulloblastoma cell lines DAOY and D341. PP2A inhibition induced an unsustainable rate of cell mitosis and resultant cell death, or “mitotic catastrophe”. PP2A inhibition also resulted in increased cellular uptake of cisplatin and decreased STAT3 signaling, likely contributing to the increased cell killing observed in response to cisplatin. This sensitization to cisplatin was also confirmed in-vivo, using a murine orthotopic medulloblastoma model.

Medulloblastoma is frequently recurrent after optimal therapy, and we created cisplatin-resistant cell lines to model this recurrence. PP2A was able to overcome this resistance to cisplatin. We also observed that the specific mechanisms of resistance to cisplatin in these cells were mediated by decreased cisplatin uptake and alteration in the estrogen-receptor pathway. Further in-vivo studies are necessary to better understand the mechanisms of cisplatin sensitization mediated by PP2A inhibition. Furthermore, our understanding of the importance of those mechanisms in medulloblastoma that lead to resistance to cisplatin and how they are overcome by LB-100 merits further study. We do, however, suggest the potential promise of this agent in better treatment of pediatric brain tumors, and hope to evaluate the efficacy of this agent in patients in the near future.

2014-2015 Medical Research Scholars Program

Scholar: Peter W. Fung
School: Harvard School of Dental Medicine
Mentor: Janice S. Lee, DDS, M.D., MS, Chief, Craniofacial Anomalies and Regeneration Section Clinical Director, NIDCR
Institute: National Institute of Dental and Craniofacial Research (NIDCR)
Research: Long-term Sequelae of Optic Nerve Encasement with Fibrous Dysplasia



Fibrous dysplasia is a developmental disorder characterized by the formation of benign bone tumors that weaken and distort normal bony architecture. These tumors often develop in the craniofacial region and frequently result in the encasement of the optic canals. Some clinicians assume that radiographic encasement by fibrous dysplasia results in gradual constriction, progressive optic neuropathy, and eventual blindness. This assumption has led some clinicians to recommend prophylactic surgical decompression to relieve constriction of the optic nerve. The validity of this premise is particularly salient for patients with normal vision since decompression surgery carries a risk of iatrogenic blindness. The aim of this study was to establish the long-term outcomes of fibrous

dysplasia encasement of the optic nerve. We performed a retrospective longitudinal study of 69 patients with fibrous dysplasia involving the lesser wing of the sphenoid bone who did not have a history of decompression surgery. All patients underwent comprehensive neuro-ophthalmologic examination and computed tomography of the skull at baseline and, at minimum, one additional evaluation. Four parameters (visual fields, acuity, color vision, and fundoscopic exam) were assessed for changes indicative of optic neuropathy, which was defined as a visual field deficit or abnormal findings in at least two of the other three parameters. This longitudinal study demonstrated that vision for the vast majority of patients remained stable without progression over an extended follow-up period. This analysis supports that prophylactic decompression of the optic nerve based solely on radiographic encasement with fibrous dysplasia is not indicated since it is not correlated with the development of visual disturbances over time.

2014-2015 Medical Research Scholars Program

Scholar: Presley Garrison
School: Brody School of Medicine at East Carolina University
Mentor: Jeffrey Baron, M.D., Head, Section on Growth and Development
Institute: Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)
Research: Spatial Regulation of Bone Morphogenetic Proteins in Postnatal Growth Plate and Articular Cartilage



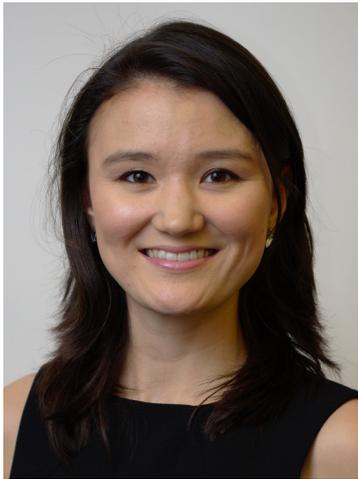
Articular cartilage pathologies are a major source of declines in health-related quality of life and increases in hospital expenditures. Osteoarthritis alone affects 33.6% of those 65 years or older in the United States. Currently, this disease is managed with pharmacotherapies that do not address the initiation or progression of the disease, but rather are intended to ameliorate pain and inflammation. A better understanding of the signaling pathways involved in cartilage development, maintenance, and degeneration will hopefully allow for a more tailored approach to prevention and early treatment of joint disease.

Growth plate cartilage and articular cartilage each have three distinct zones of chondrocytes distinguishable by histology and by transcriptome expression. Growth plate cartilage is divided into resting (RZ), proliferative (PZ), and hypertrophic (HZ) zones while articular cartilage is divided into superficial (SZ), mid (MZ), and deep zones (DZ). The bone morphogenetic protein (BMP) system serves as an important regulator of cartilage development. We previously demonstrated that a BMP signaling gradient exists across the postnatal growth plate and that this gradient serves to regulate chondrocyte differentiation. Here we hypothesized that a zonal expression gradient of BMP-related genes is also present in articular cartilage. To test that hypothesis, we used laser capture microdissection (LCM) and solution hybridization with barcoded probes (NanoString) to analyze mRNA expression of 33 BMP-related genes in all zones of growth plate and articular cartilage of 7-day old mice.

In growth plate cartilage, BMP agonists were generally expressed in HZ while BMP antagonists were primarily expressed in RZ. However, antagonists *Noggin* and *Smad7* showed greatest expression in HZ and *Gdf10* showed greatest expression in PZ. In articular cartilage, BMP agonists were primarily expressed in SZ whereas BMP antagonists were primarily expressed in MZ and DZ. Notably, BMP receptors and down-stream effectors of the BMP system showed no major differences between zones in growth plate and articular cartilage. In summary, the observed spatial expression patterns support our hypothesis that a BMP signaling gradient is present across articular cartilage such that greater BMP activity occurs in the superficial zone of the articular cartilage.

2014-2015 Medical Research Scholars Program

Scholar: Lisa Mariko Gretebeck
School: University of Pennsylvania School of Veterinary Medicine
Mentor: Kanta Subbarao, M.D., MPH, Chief, Emerging Respiratory Viruses Section, Laboratory of Infectious Diseases
Institute: National Institute of Allergy and Infectious Diseases (NIAID)
Research: Inhibition of Middle East Respiratory Syndrome Coronavirus Infection in New Zealand White Rabbits with a Human Anti-Spike Monoclonal Antibody



The Middle East Respiratory Syndrome coronavirus (MERS-CoV) is an emerging zoonotic coronavirus in humans. Identified in 2012, MERS-CoV causes acute respiratory disease associated with high mortality (36%), calling for an urgent need to develop prevention and treatment strategies. Small animal models are needed for evaluation of vaccines and antiviral drugs. Dipeptidyl peptidase-4 (DPP4), the receptor for MERS-CoV is expressed in the lungs of New Zealand white rabbits, making it possible to experimentally infect rabbits with MERS-CoV. We investigated the ability of a human monoclonal antibody (hmAb), with potent in vitro neutralizing activity against MERS-CoV, to provide protection from MERS-CoV infection in rabbits. To determine whether administration

of the MERS-CoV hmAb could prevent MERS-CoV infection, groups of four rabbits were prophylactically administered MERS-CoV hmAb in doses of either 1 mg/kg or 10 mg/kg, or a hmAb specific for Nipah-Hendra virus at 10 mg/kg as a control, by the intravenous (IV) and intranasal (IN) routes prior to intranasal administration of 10⁵ 50% Tissue Culture Infective Dose of the EMC/2012 strain of MERS-CoV. Viral RNA was measured on days one and three post-infection using quantitative reverse transcriptase-PCR (qRT-PCR) assays targeting the MERS-CoV envelope (upE) and nucleocapsid (N2 and N3) genes. Prophylaxis with the MERS-CoV hmAb via either route resulted in a significant decrease in viral RNA detection in the rabbit model. Next, to determine whether treatment with the MERS-CoV hmAb could be used to limit MERS-CoV infection, rabbits were treated with hmAb either IV or IN after viral inoculation. Antibody treatment via IV or IN routes did not result in a significant decrease in viral RNA in the lungs following infection. Our results indicate that prophylaxis with the hmAb limits viral replication and shows promise for clinical vaccine development.

2014-2015 Medical Research Scholars Program

Scholar: Molly Hague
School: University of California, San Francisco School of Dentistry
Mentor: Kenneth Yamada, M.D., Ph.D., Chief, Laboratory of Cell and Developmental Biology; NIH Distinguished Investigator
Institute: National Institute of Dental and Craniofacial Research (NIDCR)
Research: The Role of Fibronectin and Btbd7 in Oral Squamous Cell Carcinoma



Our laboratory previously established that the extracellular matrix protein fibronectin (FN) and its downstream signaling effector Btbd7 promote transient normal epithelial cell migration during development. Therefore, to help bridge the gap between cell migratory roles during development and invasive migratory behavior during cancer, we investigated the role of FN-binding integrins and Btbd7 in oral squamous cell carcinoma (SCC). Specifically, we investigated whether the FN receptor $\alpha 5\beta 1$ integrin and Btbd7 are required for oral SCC invasion and migration.

We evaluated the invasive capabilities of two oral SCC lines (SCC-9 and SCC-25), both isolated from the tongue, compared with a normal cell

line (human oral keratinocytes) using a classical Matrigel-coated transwell invasion assay. The SCC-9 cell line, but not the SCC-25 line, proved significantly more invasive than normal oral keratinocyte controls. To further evaluate the difference between the non-invasive SCC-25 cells and invasive SCC-9 cells, we investigated the role of FN in each of these lines. Immunofluorescence demonstrated that confluent SCC-9 cells assembled large FN bundles, whereas SCC-25 cells did not. Additionally, the SCC-9 cells exhibited co-localization of FN and its receptor, the $\alpha 5\beta 1$ integrin. Inhibition of $\alpha 5\beta 1$ integrin function inhibited not only SCC-9 invasion in the transwell assay, but also SCC-9 migration from spheroids in collagen. Because Btbd7 has been shown to be activated downstream of FN, we tested its role in invasiveness by depleting Btbd7 protein levels in SCC-9 cells with siRNAs. Reduction of Btbd7 levels resulted in a substantial inhibition of cell invasion through the transwells. Together, these results indicate that the FN $\alpha 5\beta 1$ integrin complex and Btbd7 play roles in SCC-9 cell invasion. Future work will seek to determine whether exogenous FN promotes Btbd7 function in these cells.

2014-2015 Medical Research Scholars Program

Scholar: Josephine Larkin Harrington
School: University of Massachusetts Medical School
Mentor: Elizabeth Murphy, Ph.D., Senior Investigator, Laboratory of Cardiac Physiology
Institute: National Heart, Lung, and Blood Institute (NHLBI)
Research: Hypertrophy-Associated Sex Differences in the Cardiac Transcriptome



Pre-menopausal females are protected from heart failure, though the mechanisms and time course over which this protection is mediated remains unclear. Cardiac hypertrophy, a major precursor to heart failure, is known to result in changes in the cardiac transcriptome, but sex-based differences are poorly understood. We examined differential messenger, micro, and long noncoding RNA expression in cardiac hypertrophy between males and females. Male and female mice each showed significant cardiac hypertrophy after receiving angiotensin II. At 2 weeks, hypertrophy and cardiac function (measured by ejection fraction) were similar between males and females. At 3 weeks, females showed significantly less cardiac hypertrophy and significantly better cardiac function than

their male counterparts, with average ejection fractions of 54% in females vs. 37% in males ($p=0.008$), and heart weights to body weights of 0.0054 in females vs. 0.0057 in males ($p=0.047$). Both healthy and hypertrophied male and female hearts had discrete expression patterns for cardiac mRNA and lncRNA.

2014-2015 Medical Research Scholars Program

Scholar: Sarah Talia Himmelfarb
School: George Washington University School of Medicine and Health Sciences
Mentor: Yasmine Belkaid, Ph.D., Chief, Mucosal Immunology Section, Laboratory of Parasitic Diseases
Institute: National Institute of Allergy and Infectious Diseases (NIAID)
Research: Site-Specific Control of T Effector Function in Human and Non-Human Primate Skin



Human skin is a large and immunologically rich organ, containing many immune cell types including more total T cells than in the blood. Yet the factors that control T effector function in human skin at steady state and in disease remain poorly understood. Our laboratory recently reported that specific commensals can uniquely and lastingly tune distinct subsets of T cells in mouse skin. We have developed novel techniques to analyze human and non-human primate (NHP) skin resident lymphocytes, enabling us to characterize these lymphocytes at steady state and investigate the relevance of the mouse model to humans and NHP. Notably, we have shown that CD3+ T cells are enriched in areas of NHP skin with high density of appendages such as hair follicles, a primary site

of commensal colonization. Fluorescence microscopy of NHP skin has revealed that T cells also preferentially localize to the epidermis and appendages, lending physiological plausibility to an interaction with commensals. In order to investigate whether commensal bacteria could play a role in shaping human skin immunity we considered the topographical diversity of the skin microbiome, and sought to determine whether T cell populations varied by site as well. We have shown that the proportion of T cells producing IL17 is enhanced in the scalp, while IL13 producing T cells are enriched in the face. Preliminary experiments also suggest that CD103, and CD69, markers of tissue residence, may differ in expression between these two sites, and this may have functional ramifications. Many diseases, including pathologies thought to be T cell mediated such as psoriasis and vitiligo vulgaris, show skin site tropism so specific that it can often be diagnostic. These findings open the door to greater understanding of the human skin immune system and may ultimately reveal potential targets for therapy of skin pathology.

2014-2015 Medical Research Scholars Program

Scholar: Richard Ho
School: Albert Einstein College of Medicine
Mentor: Peter A. Pinto, M.D., Head, Prostate Cancer Section, Urologic Oncology Branch
Institute: National Cancer Institute (NCI)
Research: Multiparametric MRI Predicts Biochemical Recurrence in Men Undergoing Radical Prostatectomy



Prostate cancer (PCa) continues to be the leading non-cutaneous cancer found in men and the second leading cancer-related cause of death. Pre-operative multiparametric magnetic resonance imaging (mpMRI) has been shown to reliably identify adverse prognostic features, including extracapsular extension (ECE) and seminal vesicle invasion (SVI). With biochemical recurrence rates (BCR) reported as high as 27% after radical prostatectomy, our goal was to evaluate the utility of preoperative mpMRI characteristics to predict BCR after surgery. A review of all patients who underwent robotic assisted radical prostatectomy (RARP) at the National Institutes of Health (NIH) identified 370 male subjects. Patients were defined as having BCR following the guidelines of the AUA Localized Prostate Cancer Update Panel report.

Of 370 patients who had RARP, 39 met criteria for BCR with a median follow up of 24 months. Upon investigating mpMRI parameters, MRI suspicion score and ECE apparent on MRI were significantly associated with BCR. Kaplan-Meier analysis demonstrated that patients with high MRI suspicion scores had increased likelihood of BCR and more rapid incidence of recurrence as compared to moderate MRI suspicion. Those with low MRI suspicious lesions did not experience BCR. These values were used to generate a nomogram that remains to be validated with a new cohort of patients. Preoperative mpMRI characteristics may aid in risk stratification, patient counseling, and modification of surgical technique in men with high risk imaging features that are concerning for BCR. The integration of mpMRI characteristics into a comprehensive nomogram for BCR may provide for improved surgical and postoperative management. Future work includes nomogram validation and continued postoperative follow-up of our patient cohort.

2014-2015 Medical Research Scholars Program

Scholar: Jason Kenneth Hsieh
School: Cleveland Clinic Lerner College of Medicine of Case Western Reserve University
Mentor: Kareem Zaghloul, M.D., Ph.D., Staff Clinician, Functional and Restorative Neurosurgery Unit, Surgical Neurology Branch
Institute: National Institute of Neurological Disorders and Stroke (NINDS)
Research: Graph Metrics of Seizure Foci in Epilepsy



Epilepsy affects approximately 50 million individuals worldwide. Many patients have disease that is refractory to medical management and suffer detrimental neurologic and socioeconomic consequences. Patients with refractory epilepsy often undergo surgery for treatment of their disease. However, successful surgical treatment requires identification of a seizure initiating zone (focus), which is a time-consuming and inexact process. Even after undergoing invasive implantation of intracranial electrodes, inpatient monitoring, and surgical resection, many patients continue to have seizures.

Foci are normally identified by epileptologists through visual inspection of electroencephalographic (EEG) traces. However, time-frequency analysis and mathematical modeling offers the ability to extract information from EEG recordings that is not immediately visible to the naked eye. Graph theory is a branch of mathematics dedicated to the study of networks, and by using graph-theoretical approaches, we sought to better characterize putative seizure-onset foci to improve or predict outcomes of epilepsy surgery.

Patients underwent implantation of intracranial electrodes for the clinical purpose of seizure localization. Intracranial EEG signals were recorded at a sampling rate of 1000 Hz and were re-referenced using a weighted common average. Graphs were constructed by windowing the EEG signal into 2.5 second windows incremented at 1 second steps, after which the average pairwise coherence in each classical frequency band was computed and used to populate adjacency matrices, resulting in one graph per second for each frequency band representing the connectivity of each pair of electrodes. Graph metrics such as degree and centrality measures were applied and putative focal electrodes were compared to non-focal electrodes at baseline and at ictal onset. Preliminarily, seizure foci demonstrated graph values that differed from non-seizure foci.

2014-2015 Medical Research Scholars Program

Scholar: Ali Jamil
School: New York University School of Medicine
Mentor: Joshua D. Milner, M.D., Chief, Genetics and Pathogenesis of Allergy Section, Laboratory of Allergic Disease
Institute: National Institute of Allergy and Infectious Diseases (NIAID)
Research: Clinical Aspects of Hyper IgE-Like Cohorts

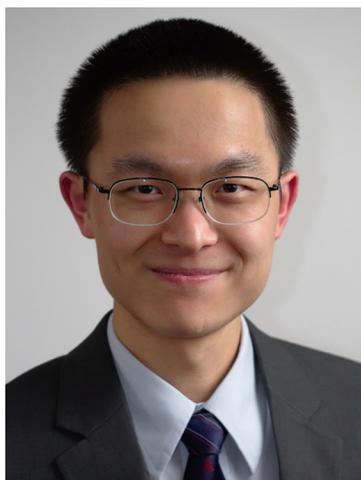


Hyper IgE Syndromes have a variety of genetic etiologies and phenotypic presentations. One example includes Signal Transducer and Activator of Transcription-3 (STAT-3) deficiency leading to Job's Syndrome, which exhibits an autosomal dominant inheritance pattern. There also exists dedicator of cytokinesis-8 (DOCK-8) deficiency syndrome, which exhibits an autosomal recessive inheritance pattern. Patients with hyper IgE syndromes experience wide variability in symptom severity, but they characteristically present with severe eczematous rash, Staphylococcal skin abscesses, recurrent pneumonia, skeletal abnormalities, and eosinophilia. We examined a cohort of patients with elevated IgE levels who presented with symptoms associated with known hyper-

IgE syndromes but failed to test positive for the known genetic abnormalities for which we screen, such as STAT-3 and DOCK-8. The primary goal of this clinical study was to identify common phenotypic profiles within this cohort of patients to potentially identify discrete disorders and novel genetic mutations. We hypothesized that one or more novel genetic mutations leading to a hyper-IgE syndrome associated with atopy were present within this cohort. This cohort was assembled from 15 different protocols at the NIH and consisted of 150 patients, 60% male and 40% female, with an age range of three to 58. Data gathering involved analysis of patient medical records in the NIH Clinical Research Investigation System and the Crimson database maintained by NIAID. Preliminary results show commonalities within the cohort, in which 60% have eczema, 32% have retained primary teeth and hyperextensible joints, and 52% experienced recurrent bacterial skin infections. Investigation into the cohort has shed light into a subgroup of pediatric patients who exhibit a unique combination of autoinflammatory and allergic features. Next steps involve compilation of the phenotypic data into a principal component analysis and sequencing of stored genomic material from all patients to identify one or more common genetic variants.

2014-2015 Medical Research Scholars Program

Scholar: Liwei Jiang
School: Johns Hopkins University School of Medicine
Mentor: Bradford J. Wood, M.D., Director, NIH Center for Interventional Oncology Chief, Interventional Radiology Section
Center: Clinical Center (CC)
Research: Techniques for the removal of embedded inferior vena cava filters



One of the more serious sequelae of deep venous thrombosis (DVT) is thrombus migration to the lungs to cause pulmonary embolism (PE), which can lead to significant disability and death. Anticoagulants are used to prevent PE in the setting of DVT, but some patients cannot receive anticoagulants for a variety of reasons, such as a recent surgery or a bleeding disorder. In these cases, an inferior vena cava (IVC) filter may be inserted to help prevent PE by capturing emboli from the lower extremity veins.

Although IVC filters are generally considered safe, leaving them in the body for a long time is risky and is associated with complications such as filter migration, device breakage, and vein perforation. The so-called retrievable IVC filters can theoretically be removed when they are no

longer needed, but filters are successfully removed in only one-third to two-thirds of cases. Over time, endothelial cells and other tissues grow around the legs of the IVC filter, making filter removal more difficult. This is a significant clinical problem.

Our team is designing and testing IVC filter removal methods. Radiofrequency (RF) ablation is a technique that uses high-frequency electrical current to burn tissue. It is increasingly used in the treatment of visceral malignancy. We can apply RF current to an imbedded, immobilizable IVC filter to burn away the tissue that traps the filter in the vena cava. In preliminary benchtop ex vivo studies, application of 100 mA of current for five seconds resulted in a 42% decrease in the force required to remove the filter (from 1.081 N to 0.632 N), with no noticeable damage to the vessel wall. Additional experiments demonstrated that higher or longer RF currents may be necessary in physiologic situations. We are conducting further studies ex vivo and in porcine models in vivo to demonstrate the safety and feasibility of this novel filter retrieval approach.

2014-2015 Medical Research Scholars Program

Scholar: Nikhil Jiwrajka
School: Johns Hopkins University School of Medicine
Mentor: Thomas A. Wynn, Ph.D., Chief, Immunopathogenesis Section, Laboratory of Parasitic Diseases Scientific Director, NIH Oxford-Cambridge Scholars Program
Institute: National Institute of Allergy and Infectious Diseases (NIAID)
Research: Evaluation of IL-13R α 2 as a Therapeutic Target for Pulmonary Fibrosis



Idiopathic pulmonary fibrosis (IPF) is a progressive and lethal interstitial lung disease that occurs in adults. Availability of the first FDA-approved therapeutic agents for IPF in late 2014 represented a tremendous advance in the field, but these agents are not curative and more effective therapies are needed. Our lab is interested in exploring immunomodulatory therapies that may mitigate persistent fibroblast activation in this setting.

Bronchoalveolar lavage (BAL) fluid of IPF patients shows increased levels of the inflammatory cytokine, IL-17A, suggesting a possible role for Th17-mediated immune responses in IPF. Recently, several groups have shown that Th17 cells express a functional IL-13 receptor, IL-

13R α 1, that downregulates production of IL-17A, suggesting that Th2 cytokines may regulate Th17 responses. Importantly, gene expression profiling studies in IPF patients have consistently revealed increased expression of an additional IL-13 receptor, IL-13R α 2, that binds IL-13 and sequesters it from the surrounding milieu. We therefore hypothesized that IL-13R α 2 may temper the Th2-mediated regulation of Th17 immunity in IPF, and that blocking IL-13R α 2 may provide therapeutic benefit.

We examined the role of IL-13R α 2 using the IL-17A-driven bleomycin-induced lung injury model. Temporal gene expression analysis in bleomycin-treated mice showed an early increase in IL-13R α 2 expression that was rapidly accompanied by increased expression of IL-17A in the absence of an early IL-13 signature. Additionally, IL-13R α 2 isolated from the BAL fluid remained unsaturated throughout the duration of the study, suggesting that IL-13R α 2 may indeed be contributing to the development of fibrosis by mitigating the potential dampening effects of IL-13 on Th17-mediated inflammation. Though our studies with IL-13R α 2 KO mice did not reveal differences in fibrosis, they contradict earlier observations regarding the role of IL-13R α 2 in fibrosis. Given the chronic and progressive nature of IPF, we are now evaluating the role of IL-13R α 2 in a more chronic animal model more closely resembling human disease.

2014-2015 Medical Research Scholars Program

Scholar: Amrita Karambelkar
School: Icahn School of Medicine at Mount Sinai
Mentor: Richard Childs, M.D., Senior Investigator, Laboratory of Transplantation Immunotherapy, Clinical Director, NHLBI
Institute: National Heart, Lung, and Blood Institute (NHLBI)
Research: Mechanistic Insights into Efficient Targeting of Ewing's Sarcoma by Ex Vivo Expanded Natural Killer Cells: Promise for Natural Killer Cell Immunotherapy



Ewing's sarcoma (EwS) is a cancer arising in the long bones of the extremities and axial skeleton, with 5-year relapse-free survival rates of 55% vs. 21% without and with metastasis. The dire prognosis of EwS presents an opportunity for exploring new therapeutic approaches for this disease.

Natural killer (NK) cells are highly cytotoxic immune cells involved in the innate defense against cancer. They kill tumor cells via induction of apoptosis through death receptors expressed on the target cell or via release of cytotoxic granules controlled by signals from activating and inhibitory germline-encoded cell surface receptors. Based on their ability to kill tumor cells, NK cells have been explored as therapeutic

agents in settings of cell-based cancer immunotherapy. Ongoing clinical trials indicate that therapy with large numbers of ex vivo expanded NK cells is safe and can induce tumor regression in subgroups of cancer patients.

Past studies have shown that EwS cells are susceptible to NK cells. Additionally, in vitro experiments have unveiled a synergistic effect of the proteasome inhibitor bortezomib and NK cell tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) on EwS. In this study, we showed that ex vivo expanded NK cells are able to rapidly and efficiently kill EwS, primarily via release of cytotoxic granules triggered by relative lack of MHC class I on EwS, as well as signaling through the NKG2D, DNAM-1, and NKp30 NK cell receptors. Furthermore, we found that bortezomib priming of EwS resulted in increased expression of the TRAIL ligand death receptor 5 (DR5) and synergized with recombinant human TRAIL (rhTRAIL) to kill EwS. However, bortezomib pre-treatment did not result in higher NK cell lysis of EwS at four hours. This study gives us a better understanding of specific mechanisms governing NK cell targeting of EwS, which can potentially be harnessed for NK cell immunotherapy against Ewing's sarcoma.

2014-2015 Medical Research Scholars Program

Scholar: Jane Kim
School: University of California, San Diego, School of Medicine
Mentors: H. Nida Sen, M.D., MHS, Director, Uveitis and Ocular Immunology Fellowship Program;
Robert B. Nussenblatt, M.D., MPH, Chief, Laboratory of Immunology
Institute: National Eye Institute (NEI)
Research: Incidence and Prevalence of Childhood Uveitis



Uveitis can cause devastating, permanent damage to intraocular structures and can result in visual impairment. Uveitis accounts for about 10% of major visual loss or approximately 30,000 new cases of blindness in the U.S. each year (Nussenblatt, 1990). Nevertheless, uveitis is rare, with an incidence of 52.4/100,000 person-years and a prevalence of 115.3/100,000 persons (Gritz and Wong, 2004), and is known to be even more rare in the pediatric population. Thus, population-based studies are integral to gaining a better understanding of the clinical characteristics and general trends of a rare disease such as uveitis. While there are four landmark studies investigating the incidence and prevalence of uveitis in different subsets of the U.S. adult population, there are no population-

based studies examining the incidence and prevalence of pediatric uveitis in the U.S. There is, however, one Finnish population-based pediatric uveitis study incorporating data collected in the 1980s (Päivönsalo-Hietanen et al., 2000); unfortunately, the study's findings have been difficult to generalize to other countries with more racially diverse populations, such as the U.S. In the present study, we aimed to determine the incidence and prevalence of childhood uveitis in the U.S. and to describe its clinical characteristics through a retrospective review of medical records at Children's National Medical Center from January 2010 to December 2011. Review of preliminary data showed evidence of a greater proportion of infectious cases than was previously reported and reflected larger numbers and greater diversity of immigrant populations in the Washington, D.C. metropolitan area than in Finland. We are currently working with collaborators in the United Kingdom to conduct a similar study, where we plan to utilize a nationalized healthcare system to more accurately determine the incidence, prevalence, and general clinical characteristics of childhood uveitis on a population level.

2014-2015 Medical Research Scholars Program

Scholar: R. Spencer Kirkland, M.D.
School: University of Missouri School of Medicine
Mentors: Kevin Camphausen, M.D., Chief, Radiation Oncology Branch; Andra Krauze, M.D., Staff Clinician Radiation Oncology Branch
Institute: National Cancer Institute (NCI)
Research: Radiation Therapy Treatment Planning in Early Stage Lymphoma



The effect of recently released international guidelines for involved-node (IN) and involved-site (IS) radiation therapy (RT) on organs-at-risk (OAR) dosimetry in patients with Hodgkins and non-Hodgkins lymphoma is unclear. The purpose of this study was to compare the dose to heart, lungs, and breast in patients with mediastinal lymphoma treated with IS, IN, and involved-field (IF) RT, as recommended in the 2014 International Lymphoma Radiation Oncology Group Guidelines.

Eleven patients (five female) with mediastinal lymphoma restricted to involvement above the diaphragm, who also had pre-chemotherapy imaging studies available, were included in this analysis. IS, IN, and IF plans were developed

for each patient. All patients were planned with a parallel opposed field technique. Percentages of OAR receiving 30 Gy (V30), 20 Gy (V20), and 5 Gy (V5), mean OAR dose, clinical target volume (CTV), volume receiving 100% dose (V100%), and volume receiving 95% dose (V95%) were recorded.

Mean ISRT volumes were non-significantly reduced compared to INRT volumes, including CTV (reduced by 99.8 cm³, p=0.06), V100% (reduced by 395 cm³, p=0.17), and V95% (reduced by 386 cm³, p=0.054). Compared to INRT, ISRT showed no significant change in mean V30, V20, V5, and mean organ dose of heart (-7.4%, -6.2%, -7.6%, -2.7 Gy), lung (-3.6%, -4.1%, -5.7%, -1.8 Gy), or breast (+2.4%, +4.4%, +2.7%, +0.8 Gy). Compared to IFRT, ISRT significantly reduced the V30, V20, V5, and mean organ dose of lung (-11%, -12%, -14%, and -4.7 Gy; all p≤0.02), with non-significant decreases of heart (-12%, -13%, -14%, -4.5 Gy) and breast (-3.4%, -3.7%, -5.2%, and -1.9 Gy). Mean CTV volume, volume coverage, and dose to OAR were not significantly different between INRT and ISRT, but ISRT improved OAR dosimetry in comparison to IFRT. Treatment planning in the absence of pre-chemotherapy scans and overreliance on clinical information as a surrogate may result in altered CTV delineation in ISRT.

2014-2015 Medical Research Scholars Program

Scholar: Jeyan Sathia Kumar
School: Chicago Medical School Rosalind Franklin University of Medicine & Science
Mentor: Michael Gottesman, M.D., Chief, Laboratory of Cell Biology, Head, Multidrug Resistance Section, NCI, Deputy Director for Intramural Research, NIH
Institute: National Cancer Institute (NCI)
Research: Bioluminescent Imaging of ABCG2 Efflux Activity at the Blood-Placenta Barrier



Many organs in the body are protected by specialized barriers, which create a controlled environment for their respective organs, such as the brain and placenta. The brain's barrier is termed the blood brain barrier (BBB) and its main function is to keep out unwanted materials. Although this system works well to protect our central nervous system from toxins and infection, it presents a significant obstacle for drug development and in administering therapeutics to the brain. A great deal of research has been done on the BBB but our understanding of how the BBB changes in diseases such as cancer is still not completely understood.

Cancer metastases to the brain are the most common type of brain tumor, and the mechanisms by which cancer cells bypass the BBB to seed the brain have yet to be elucidated. To attempt to understand the barrier, we utilized bioluminescence imaging (BLI) to observe how the barrier changed in an in vivo metastatic prostate tumor mouse model.

With the discovery that luciferin is a specific substrate for the efflux transporter ABCG2 at the BBB, we hypothesized that any change in the barrier would alter luciferin entry into the brain. We used a luciferase expressing prostate cancer cell line carrying a specific Ras mutation found to increase its metastatic tumor potential to the brain. The cells were injected into the left ventricle of mice to create a metastatic tumor model with BLI and MRI once a week for four weeks. We found that by inhibiting ABCG2 with a small molecule inhibitor, Ko143, we were able to detect tumors by BLI much sooner than by MRI. In addition we were able to demonstrate that BLI before and after ABCG2 inhibition can potentially be used as a read out of when the BBB is compromised. More data are needed to optimize these imaging modalities to analyze functional pathways in the barrier.

2014-2015 Medical Research Scholars Program

Scholar: Jennie D. Leikin
School: Tufts University School of Dental Medicine
Mentor: Bríd Ryan, Ph.D., MPH, Stadtman Investigator, Laboratory of Human Carcinogenesis, Center for Cancer Research
Institute: National Cancer Institute (NCI)
Research: Analysis of IsomiRs in Lung Cancer



MicroRNA(miRNA) are small non-coding RNA that target messenger RNA (mRNA) transcripts to modulate gene expression; they generally target the 3' UTR region of mRNA transcripts. miRNA deregulation and signatures have been correlated with progression of malignant disease and thus the possibility of clinical use for miRNAs in cancer control is the focus of much recent study. Post-transcriptional editing of miRNA contributes to the formation of miRNA isoforms known as isomiRs. Because miR-21 is overexpressed in multiple malignancies and the endoribonucleases DICER and DROSHA are known to be mutated in up to 10% of human lung cancers, we hypothesized that there is an increase in miR-21 isomiRs in tumor tissue compared with non-involved tissue. TCGA exome

sequencing data for miR-21 in both lung adenocarcinoma (n=420) and squamous cell carcinoma (n=258) were extracted and analyzed using STATA 13. We observed the presence of significantly more isomiRs of miR-21 in both lung adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC) compared to non-involved adjacent lung tissue. LUAD had total of 244 5p variants and LUSC had 232 5p variants, compared with 74 x and 105 y in non-involved tissue, respectively. The most prevalent isomiR of miR-21 was not the canonical isomiR and the most common edit was a 5' deletion and 3' addition. In both cancer types, 43 miR-21-5p isomiRs that were prevalent (defined as detected in more than 50% of patients) had a significantly higher abundance in tumor versus non-involved tissue samples (p<0.05). The average fold change was XYZ11.37 in LUAD. Of the 43 prevalent isomiRs, 28 isomiRs had a change in the canonical miR-21 seed sequence. An analysis of the targets of these isomiRs using Targetscan and Diana revealed variation in gene targets which ranged from 2% to 87% of canonical miR-21 targets. Laboratory studies are required to determine the functionality of these isomiRs and their relevance for human cancer diagnosis and treatment.

2014-2015 Medical Research Scholars Program

Scholar: Angela Liu
School: University of California San Diego, School of Medicine
Mentor: Nicolas Wentzensen, M.D., Ph.D., Senior Investigator, Epidemiology and Genetics Section, Hormonal and Reproductive Epidemiology Branch
Institute: National Cancer Institute (NCI)
Research: Reproducibility and Determinants of Colposcopic Impressions and Predictive Diagnostic Accuracy



Each year in the U.S., about 3 million women are evaluated with colposcopy for abnormal cervical screening results. However, evidence from prior research has suggested that the diagnostic accuracy of current colposcopy practice is suboptimal. In addition, the use of static images of the cervix in teaching colposcopy has been criticized because they do not capture the dynamic, time-sensitive aceto-whitening changes in the cervical epithelium.

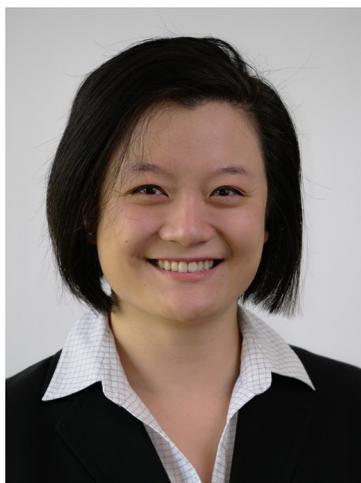
To address the deficiencies of the current practice and teaching of colposcopy, we designed The Biopsy Study, an observational study of 690 women (18 years and older) referred for colposcopy after abnormal cervical screening results at Oklahoma University.

A comprehensive colposcopy-biopsy protocol ensured highly sensitive disease ascertainment. Comparing the inter-rater agreement between live colposcopic impressions and impressions by static image assessment, we showed the subjective discrimination of low-grade from less severe impressions depends on the mode of live versus static visualization of the cervix ($p < 1 \times 10^{-6}$). However, the subjective discrimination of high-grade from less severe impressions is not dependent on the mode of visualization (p -value 0.07). Knowing the patients' high-grade referral cytology biases the colposcopic assessment by upgrading low-grade to high-grade impressions ($p = 1.3 \times 10^{-4}$), which improves the detection sensitivity for HSILs at the biopsy threshold of high-grade colposcopic impressions ($p = 5 \times 10^{-3}$). Live colposcopy is more sensitive at detecting HSILs than static image assessment at the biopsy threshold of low-grade impressions, but not aceto-whitening. HSIL detection is comparable at the biopsy threshold of high-grade impressions on live colposcopy versus static image assessment.

Our results support the validity of using static images of the cervix for teaching normal and aceto-whitening impressions; diagnostic accuracy of high-grade colposcopic impressions improves with consideration of increased cervical disease risk from high-grade cytology results. Remote expert consultation based on static images may facilitate diagnosis of cervical cancer pre-cancers in low-resource, usually high-disease-burden settings. However, the teaching of diagnostically accurate assessment of low-grade impression continues to require apprenticeship with an experienced colposcopist.

2014-2015 Medical Research Scholars Program

Scholar: Victoria Ting-Yi Liu
School: University of California, San Diego, School of Medicine
Mentor: Mark Hallett, M.D., Chief, Human Motor Control Section
Institute: National Institute of Neurological Disorders and Stroke (NINDS)
Research: Decreased Heart Rate Variability in Functional Movement Disorders



Autonomic activity is of interest in functional movement disorders (FMD) given the hypothesis that the abnormal movements seen in FMD are in part the result of converted psychological stress and increased vulnerability to emotional reactivity. Studies have shown that the autonomic nervous system participates in self-regulation of emotion. Heart rate variability (HRV) provides a quantitative assessment of central autonomic activity. Reduced HRV can be reflective of autonomic dysregulation, typically with decreased parasympathetic input relative to sympathetic input. While reduced HRV has been demonstrated in a variety of mood and anxiety disorders, autonomic regulation has not been well studied in the conversion disorder population.

Patients with “clinically definite” FMD and age- and gender-matched healthy controls were hospitalized overnight for continuous electrocardiogram (ECG) recording. ECG data was analyzed using Impresario software, artifacts were removed, and data were imported into Matlab for calculation of multiple HRV time and frequency domain measures. HRV parameters analyzed included standard deviation of the N-N interval (SDNN), square root of the mean of the sum of the squares of differences between adjacent R-R intervals (RMSSD), and power spectral density analysis in very low frequency (VLF), low frequency (LF) and high frequency (HF) ranges. Patients on heart rate altering medications or with history of cardiovascular disease were excluded.

FMD patients demonstrated reduced HRV compared to controls, as determined by significantly diminished RMSSD ($p=0.02$). They also showed increased index of sympathetic to parasympathetic activity compared to healthy controls, as indicated by significantly increased LF/HF ratio ($p<0.05$). Incidentally, FMD patients also demonstrated increased mean heart rate ($p<0.01$). Alterations in regulation of the autonomic nervous system may play a significant role in the pathophysiology of FMD and merit further exploration.

2014-2015 Medical Research Scholars Program

Scholar: Dino Maglic
School: Brody School of Medicine at East Carolina University
Mentor: William Gahl, M.D., Ph.D., Senior Investigator, Medical Genetics Branch, Clinical Director, National Human Genome Research Institute, Director, NIH Undiagnosed Diseases Program
Institute: National Human Genome Research Institute (NHGRI)
Research: Genetic Modeling in Zebrafish



Use of zebrafish as experimental models has gained popularity in studying human development and disease. Gene editing techniques and the practicality of use of these vertebrates has made zebrafish the ideal organism in which to study genetic diseases. GNE myopathy is a rare adult onset myopathy due to defects in an essential enzyme required for sialic acid biosynthesis. That enzyme, UDP-GlcNAc 2-epimerase/ ManNAc kinase, is encoded by the GNE gene whereas sialic acids are terminal sugars which are known to decorate glycoproteins and glycolipids. Decreased enzyme activity results in decreased production of sialic acids and therefore decreased sialylation of these macromolecules.

In collaboration with the NHGRI (National Human Genome Research Institute) Zebrafish Core facility, we have been able to create models that harbor gne loss of function alleles by different gene editing techniques. By injection of Morpholino antisense oligonucleotides, we can inhibit splicing of the gene resulting in a gne zebrafish model with a severe muscle phenotype. Knock out of the gene by CRISPR-CAS (clustered regularly interspaced short palindromic repeats) methods also led to a severe muscle phenotype and embryonic lethality. Creation of a germline missense mutation near the end of the gene using the ENU (N-ethyl-N-nitrosourea)/TILLING (Targeting Induced Local Lesions IN Genomes) method resulted in a zebrafish model with a disorganized muscle fiber pattern and decreased sialic acid staining on microscopy. Importantly, these fish survive into adulthood, making them a good model for adult onset GNE myopathy. Administration of a nontoxic sialic acid precursor, N-acetylmannosamine, results in partial rescue of the phenotype of these fish. These models have been the basis for ongoing clinical trials using sialic acid therapies to recover muscle atrophy and weakness in these patients. The pathophysiology of this disease is still unknown and we plan to use these models to better understand the disease mechanism, identify biomarkers and genetic modifiers, and further develop therapy.

2014-2015 Medical Research Scholars Program

Scholar: Trisha Pasricha
School: Vanderbilt University School of Medicine
Mentor: Thomas Wynn, Ph.D., Chief, Immunopathogenesis Section, Laboratory of Parasitic Diseases; Scientific Director, NIH Oxford-Cambridge Scholars Program
Institute: National Institute of Allergy and Infectious Diseases (NIAID)
Research: The Role of the IL-13 Decoy Receptor in Inflammatory Bowel Disease



In refractory inflammatory bowel disease (IBD), approximately one-third of patients do not respond to administration of anti-TNF-alpha agents, and an additional one-third ultimately lose their initial response. There is a clinical need to explore broader therapeutic options within the inflammatory signaling pathways that govern immune dysregulation. We therefore sought to explore the properties of the IL-13Ra2 decoy receptor in a mouse model of IBD. The wound healing and anti-inflammatory effects of IL-13 are dampened by its non-signaling decoy receptor, IL-13Ra2. We hypothesized that genetic deletion of the decoy receptor might augment the protective role of IL-13 and restore immune homeostasis in IBD. Toward this end, we examined the development of IBD

in mice exhibiting a genetic deletion of the IL-13 decoy receptor. We demonstrated that IL-13Ra2 knockout (KO) mice demonstrated less weight loss, improved pathology, and reduced induction of inflammatory cytokines in colonic tissue compared to wild-type mice in a chronic IBD model. Our findings showed for the first time that genetic deletion of the IL-13Ra2 receptor in mice can serve a protective role in a chronic IBD model across several physiological parameters. Furthermore, we explored the interactions of the IL-13 decoy receptor in *in vitro* studies using human intestinal epithelial fibroblasts obtained from patients with IBD. The IL-13 decoy receptor was found to be upregulated in these cells when stimulated by TNF-alpha and IL-17, both implicated in the pathogenesis of IBD. Our data suggests blockade of the IL-13 decoy receptor may be an important therapeutic approach for patients with IBD by restoring the normal homeostatic function of IL-13.

2014-2015 Medical Research Scholars Program

Scholar: Martin G. Piazza
School: Wake Forest School of Medicine
Mentor: Prashant Chittiboyna, M.D., Staff Clinician, Surgical Neurology Branch
Institute: National Institute of Neurological Disorders and Stroke (NINDS)
Research: Development of a Novel Vasogenic Brain Edema Model in Rats



Peritumoral vasogenic brain edema (PTVE) is a significant cause of morbidity and mortality in patients with a variety of brain tumors. If left untreated, it may result in brain herniation and death. Vasogenic edema results from chronic inflammation or factors released by tumors causing blood brain barrier breakdown and interstitial fluid accumulation from bulk flow from the intravascular space. Further study of PTVE would be useful in elucidating the mechanisms of current treatment options for PTVE, specifically dexamethasone, and potentially finding targets for therapies with fewer side effects. While several models exist for cytotoxic edema, the mechanism of this type of edema is vastly different from that of vasogenic edema. As such, the current brain

edema models that exist are not useful in studying PTVE. Previous work has shown that infusion of vascular endothelial growth factor (VEGF), an angiogenic factor commonly released by tumors, causes resultant blood brain barrier (BBB) disruption. BBB breakdown is a major component of the pathogenesis of vasogenic brain edema. Rat striata were infused with a solution of VEGF and rat serum albumin (RSA) as a carrier protein at 1ul/hr using a stereotactically placed brain infusion cannula. MRI scans were performed at 48 hrs and day 6 of infusion. Upon euthanization, brains were frozen for histological confirmation of edema and BBB breakdown using H&E staining, as well as CD34, CD31, GFAP, and ZO-1. MRI data were processed and analyzed using software routines written in MATLAB and Osirix. T2 weighted scans showed reproducible hyperintensity along the external capsule in experimental animals compared to control animals, signifying fluid accumulation. Additionally, juxtacannular contrast enhancement on T1 was seen only in the experimental group. Using both the histologic studies and MRI imaging, reproducible results confirmed the creation of a vasogenic brain edema model in rodents to be used in future studies.

2014-2015 Medical Research Scholars Program

Scholar: Paul Kirkpatrick Reardon
School: Columbia University College of Physicians and Surgeons
Mentor: Armin Raznahan, M.D., Ph.D., Chief, Developmental Neurogenomics Unit, Child Psychiatry Branch
Institute: National Institute of Mental Health (NIMH)
Research: Subcortical Morphometrics in Sex Chromosome Aneuploidy



The risk profile for neurological and psychiatric disease is different for men and women. Men are four times as likely to be diagnosed with autism, and women have a two-fold greater risk for major depressive disorder. The origins of this risk differential may reside in the brain, where the biological determinants of sex, the X- and Y- chromosome and sex steroids, have been demonstrated to cause divergent global volumetric effects. However, less is known about the normative sex differences in subcortical brain regions closely associated with behavioral disorders. Our project took a two-pronged approach to interrogate the underlying causes of sex differences in the brain. First, we mapped the normative neuroanatomical differences in men and women in the striatum, thalamus,

and globus pallidus. Second, we hypothesized that increased sex gene dosage seen in various sex chromosome aneuploidy (SCA) syndromes would exaggerate the normative differences.

Using a novel segmentation method for parcellation of the subcortex, we examined 100 adolescent brains from a rare cohort of youth with SCA (23 XXX, 38 XXY, 19 XYY, 15 XXYY, 5 XXXXY), and 184 typically developing control subjects (87 XX, 97 XY). To place the anatomical findings within a normative framework, we used a second independent sample of typically developing youth (37 XY, 33 XX) to create an allometric scaling law of volumetric shifts for the subcortex, given changes in total brain volume. We found sex normatively decreases total brain volume to a greater extent than the volumes of the striatum, thalamus, and pallidum. While SCA caused global volumetric reductions, the reductions seen in the subcortex were heterogeneous, with the pallidum displaying greatest volume loss ($p < 2.2 \times 10^{-16}$). When placed into the context of normative allometric volume relationships, only the pallidum showed losses above and beyond what would be expected given the normative link between subcortical and global volumes.

2014-2015 Medical Research Scholars Program

Scholar: Joseph Cataquiz Rimando
School: Medical College of Georgia at Georgia Regents University
Mentor: Daniel Fowler, M.D., Senior Investigator; Head, Cytokine Biology Section, Experimental Transplantation and Immunology Branch
Institute: National Cancer Institute (NCI)
Research: Amino Acid Excess Modulates the Phenotype of Rapamycin-Resistant CD4+ T Cells



Allogeneic hematopoietic stem cell transplantation is a critical therapy for otherwise incurable hematologic malignancies. This therapy provides beneficial graft-versus-tumor (GVT) effects but is limited by graft-versus-host (GVH) disease. Donor T cells within the transplant mediate both of these effects. In murine models and clinical trials, our group has shown that ex vivo-manufactured rapamycin-resistant donor CD4+ T cells provide a favorable balance of GVT and GVH effects.

Recently, we have found that rapamycin-resistant T cells of the T helper 2 (Th2) subset cultured in an excess of essential and non-essential amino acids are modulated towards a central memory (CD45RO+CCR7+CD62L+) phenotype. Central

memory T cells are may be highly beneficial for cellular therapy, as these cells persist within the body for long periods of time and rapidly proliferate in response to antigen. Additionally, these amino acid-modulated T cells have increased mitochondrial mass but poor mitochondrial membrane stability. These cells have increased production of the immature Th2 cytokines IL-2 and IL-4 and greatly reduced production of the mature Th2 cytokines IL-5 and IL-13. This cytokine profile is consistent with the central memory phenotype. In terms of the mechanism behind this phenomenon, this excess of amino acids appears to shut down the mTOR pathway and its downstream targets S6K, AKT, and 4E-BP1. Blockade of the mTOR pathway leads to increased expression of KLF2, a critical transcription factor controlling expression of the central memory phenotype. These findings provide an exciting avenue to improve cellular therapies for cancer patients.

2014-2015 Medical Research Scholars Program

Scholar: Taufiq Salahuddin
School: Wake Forest University School of Medicine
Mentor: Nehal Mehta, M.D., MSCE, Lasker Clinical Research Scholar, Section of Inflammation and Cardiometabolic Diseases
Institute: National Heart, Lung, and Blood Institute (NHLBI)
Research: Psoriasis as a Model to Study Inflammation and Cardiometabolic Diseases



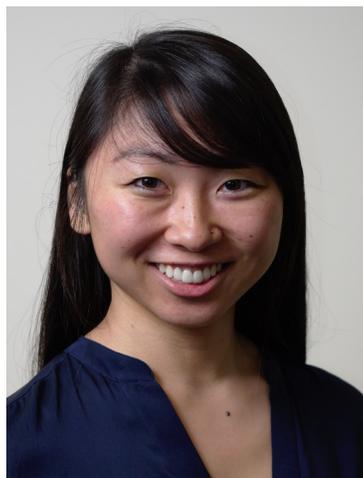
Though classically thought of as a skin disease, we now know that skin lesions in psoriasis, a chronic autoimmune inflammatory disease, are just one manifestation of a multiorgan disorder. Underlying vascular and metabolic dysfunction predisposes patients with psoriasis to myocardial infarction and stroke. Th1 and Th17 cells are highly activated during flares of psoriasis, and these same cells have been implicated in atherogenesis. Therefore, we propose that psoriasis might serve as a good *in vivo* model to study the effects of chronic inflammation on the vascular system.

Vascular inflammation, as measured by aortic uptake of 18-fluorodeoxyglucose (FDG) on positron emission tomography/computed tomography (PET/CT), is increased in patients with psoriasis compared with non-psoriasis control subjects. Further, patients with psoriasis have metabolic dysfunction, characterized by increased insulin resistance and decreased cholesterol efflux capacity, which is increasingly recognized as more important than high density lipoprotein concentration alone in protection against and prediction of cardiac risk. However, the relationship of psoriasis and its associated findings with atherosclerotic plaque burden in the coronary arteries is unknown.

We hypothesized that accelerated formation of inflammatory, lipid-rich, noncalcified plaque in the coronary arteries is responsible for the increased cardiac risk in psoriasis. Patients were recruited as part of an ongoing 4-year prospective cohort study of cardiometabolic disease in psoriasis. We utilized Coronary Computed Tomography-Angiography (CCTA) to visualize and quantify the amount of plaque in the coronary vessels. We have found in regression analyses that vascular inflammation was associated with increased coronary atherosclerotic burden, especially noncalcified plaque, even after adjustment for cardiovascular risk factors ($\beta^*=0.42$, $p<0.001$; $\beta=0.39$, $p<0.001$, for total and noncalcified burden, respectively). We also found noncalcified plaque burden decreased as cholesterol efflux capacity increased ($B^*=-0.33$, $p<0.001$, $B^*=-0.24$, $p<0.001$). This type of study helps us better understand inflammation-mediated atherogenesis and may lead to discovery of new targets for interventions in atherosclerosis.

2014-2015 Medical Research Scholars Program

Scholar: Kei Satoh
School: Icahn School of Medicine at Mount Sinai
Mentor: Electron Kebebew, M.D., FACS, Chief, Endocrine Oncology Branch
Institute: National Cancer Institute (NCI)
Research: Identification of Niclosamide as a Novel Anti-cancer Agent for Adrenocortical Carcinoma



Adrenocortical carcinoma (ACC) is a rare and highly aggressive disease. ACC can either be hereditary or sporadic, and has been associated with IGF2, b-catenin, and p53 mutations. The 5-year survival rate of ACC is 18-36%. Surgery remains the only curative therapy, with other therapeutic options providing only limited benefit. The discovery of effective agents for ACC is necessary to improve the prognosis of patients with ACC. Drug repurposing is an emerging approach to identify new indications for existing drugs, and could provide promising new agents for ACC. The objective of this study was to use quantitative high throughput screening (qHTS) to identify agents with antineoplastic activity against ACC cell lines and validate these findings through in vitro studies. Screening of 3826

compounds was performed on three established ACC cell lines, BD140A, SW-13, and NCI-H295R. Twenty-one active compounds were identified with an efficacy of >80% in all three cell lines. Niclosamide, an anti-helminthic drug used in humans for over five decades, was selected for further validation. Niclosamide anti-helminthic activity results from uncoupling of oxidative phosphorylation and inhibition of ATP production, while studies of Niclosamide antineoplastic activity in other cancer types have found that it inhibits cellular pathways including wnt/b-catenin, Akt/mTor, Notch, and STAT3. Niclosamide was found to inhibit cellular proliferation in all 3 ACC cell lines at concentrations below physiologically attainable C_{max} through induction of caspase-dependent apoptosis and G1 cell cycle arrest. Additionally, treatment with Niclosamide inhibited expression of b-catenin and Akt. Finally, treatment with Niclosamide resulted in depolarization of the mitochondrial membrane potential. These findings suggest that Niclosamide has antineoplastic activity against ACC through inhibition of multiple cellular pathways as well as alterations in cellular metabolism, and may be a promising new agent for the treatment of ACC.

2014-2015 Medical Research Scholars Program

Scholar: Jack Williams Shteamer
School: Emory University School of Medicine
Mentor: Wade Chien, M.D., Staff Clinician, NIDCD; Assistant Professor, Department of Otolaryngology-Head & Neck Surgery, Johns Hopkins School of Medicine
Institute: National Institute on Deafness and Other Communication Disorders (NIDCD)
Research: Gene Therapy in the Inner Ear



The vestibular system of the inner ear is involved with maintaining balance. Dysfunction of the vestibular system is a debilitating medical condition, with as many as 40% of US adults seeking medical treatment for dizziness sometime in their lives. Currently there are few effective treatments. However, gene therapy has recently been proposed as a possible treatment.

Usher syndrome is the most common form of hereditary deafness and blindness. In many patients, vestibular dysfunction is also present. Mutations in the gene for whirlin are associated with Usher syndrome. Whirlin is necessary for the development of stereocilia on the apical surface of hair cells in the inner ear. Mutations cause abnormal and dysfunctional stereocilia,

which results in hearing loss and vestibular dysfunction.

The whirler mouse, a model of hereditary hearing loss and vestibular dysfunction, has a mutation in the whirlin gene. These mice have short stereocilia, are deaf, and display circling behavior associated with vestibular dysfunction. The focus of our research is to assess whether gene therapy can restore vestibular function in the whirler mouse. To answer this question, we injected adeno-associated virus (AAV8) containing wild-type whirlin cDNA into the posterior semicircular canal of neonatal whirler mice. One month later, we assessed their vestibular function, hair cell morphology, and whirlin expression. Initial results showed that injection of 0.49 μ L of AAV8-whirlin into the posterior canal resulted in a statistically significant increase in the length of hair cell stereocilia in the utricle. This led to recovery of vestibular function in 3 of 11 whirler mice (27%). When 0.98 μ L of virus was injected, 4/5 mice responded to the therapy (80%). Further study will focus on using a more precise measure of vestibular function, and on elucidating the developmental timeframe in which the gene therapy is effective.

2014-2015 Medical Research Scholars Program

Scholar: Rebecca Ann Sorber
School: Indiana University School of Medicine
Mentor: Udo Rudloff, M.D., Ph.D., Head, Gastrointestinal Oncology Section,
Thoracic and Gastrointestinal Oncology Branch
Institute: National Cancer Institute (NCI)
Research: Immunomodulatory Peptide RP-182 in Pancreatic Adenocarcinoma



Tumor-associated macrophages (TAMs) possess an alternatively activated, immunosuppressive phenotype (M2) and have previously been implicated in accelerated tumor progression and poor outcomes in pancreatic cancer. RP-182 is a synthetic, 10 amino acid peptide shown via in silico modeling to likely target TAM-specific surface receptors. Based on its potential immunomodulatory properties, we tested whether RP-182 exhibits tumor suppressive activity in a xenograft model of pancreas cancer. Athymic nude mice were xenografted with human pancreas (HPAC) cancer cells and treated with either vehicle (normal saline), gemcitabine, RP-182, or a combination dosed twice weekly via intraperitoneal injection. Following tumor and spleen excision, CD11b+ cells were isolated.

CD11b+ cells were co-cultured with HPAC cells at varying concentrations and survival of cancer cells was quantified. Polarization of CD11b+ cells was measured via qRT-PCR analysis using previously validated markers for murine M1 and M2 phenotypes. Animals treated with RP-182 alone and combination RP-182 plus gemcitabine showed significant suppression of tumor growth when compared to animals in the vehicle-treated and gemcitabine-alone groups, respectively ($p=0.007$ and $p<0.001$). HPAC cells co-cultured with CD11b+ cells isolated from RP-182 treated animals showed $\geq 50\%$ less viability after 72 hours than did cells co-cultured with CD11b+ cells from vehicle-treated animals ($p=0.069$ via 2-way ANOVA). Analysis via qRT-PCR showed upregulation of the M1-selective marker iNOS (>13 -fold) in the RP-182 treated tumors as the most significantly altered marker between the two groups ($p=0.02$). Overall, RP-182 demonstrates tumor suppressive activity and minimal toxicity in a xenograft model of pancreatic cancer both as a stand-alone treatment and in combination with gemcitabine. Decreased survival of pancreas cancer cells following coculture with CD11b+ cells isolated from RP-182 treated mice versus controls suggests increased tumoricidal activity in macrophages exposed to RP-182. Likewise, transcriptional changes confirmed via qRT-PCR suggest that treatment with RP-182 alters polarization of tumor-associated macrophages toward an M1 phenotype.

2014-2015 Medical Research Scholars Program

Scholar: Amol Sura
School: LSUHSC School of Medicine, New Orleans
Mentor: Robert Nussenblatt, M.D., MPH, Chief, Laboratory of Immunology
Institute: National Eye Institute (NEI)
Research: Scavenger Receptor A Alters Experimental Autoimmune Uveitis



Autoimmune uveitis accounts for approximately 10% of blindness in the US. Antigen presenting cells (APCs), such as macrophages and dendritic cells, are pivotal drivers of tissue damage in uveitis. A well-established pathway with which APCs organize the immune response is through a set of receptors known as scavenger receptors, of which scavenger receptor-A1 (SR-A1) is a prototypical example. Scavenger receptor-A1 plays a role in a number of diseases, such as atherosclerosis, cancer, and Alzheimer's disease, but its effect on uveitis is not well understood.

Using a mouse model for uveitis, we investigated the effects of SR-A1 on uveitis and APC function. Deleting SR-A1 decreased the severity of autoimmune uveitis, as detected by clinical

fundoscopy and histological examination ($p < .01$ for both). Mechanistic studies revealed attenuated expression of the co-stimulatory markers CD80, CD86, I-Ek, and CD40 in SR-A1 knockout macrophages and dendritic cells ($p < .01$). SR-A1 knockout macrophages also showed lower RNA expression levels of canonical pro-inflammatory cytokines (TNF- α , IL-1B, and IL-6) compared to wild type macrophages. Finally, knocking out scavenger receptor-A1 reduced antigen-specific lymphocyte proliferation in response to uveitogenic antigen, reflecting a lower level of immunologic memory in SR-A1 knockout mice. These alterations show that knocking out SR-A1 protects mice from uveitis and reduces activation of both innate and adaptive immunity.

2014-2015 Medical Research Scholars Program

Scholar: Alisa Thavikulwat, M.D.
School: University of Rochester School of Medicine and Dentistry
Mentors: Emily Chew, M.D., Deputy Director, Division of Epidemiology and Clinical Applications; Brett Jeffrey, Ph.D., Staff Scientist, Ophthalmic Genetics and Visual Function Branch
Institute: National Eye Institute (NEI)
Research: Innovations in the Diagnosis and Management of Macular Diseases



Diseases affecting the retinal pigment epithelium (RPE) layer of the retina, such as age-related macular degeneration (AMD) and Stargardt disease are a leading cause of blindness in the world. Current methods of evaluating the RPE do not indicate visual function of the RPE, so we developed a non-invasive method of quantifying RPE changes in maculopathy for potential application in clinical diagnosis and as an outcome measure for clinical trials. We designed a focal electro-oculogram (EOG) using a light stimulus and tested our protocol on the eyes of 15 healthy volunteers. We found that a 40° stimulus at 40 cd/m² consistently generated a light rise produced by the RPE cells and that this response was driven by cells in our target area of the retina. The next step in the project will be

to test the focal EOG in a patient with focal macular disease to show that it can pick up early changes that are undetectable with the traditional full-field EOG.

In neovascular AMD, treatment with anti-VEGF therapy is well-documented to preserve vision, but there is concern that aggressive anti-VEGF therapy may increase the risk of developing geographic atrophy (GA). We evaluated the incidence and progression of 75 subjects enrolled for up to 5 years in a prospective clinical study assessing the effects of ranibizumab therapy in eyes with neovascular AMD. About 20% of subjects developed new GA in their study eye, all in areas of patchiness on fundus autofluorescence photography, indicating RPE disease that may have predisposed those eyes to developing GA. Rates of GA growth were similar between study and untreated and non-neovascular fellow eyes. These results highlight the importance of multi-modal imaging in the management of neovascular AMD, but further study is needed to determine whether these eyes were destined to develop GA regardless of anti-VEGF therapy.

2014-2015 Medical Research Scholars Program

Scholar: Denise Fay Ye
School: Vanderbilt University School of Medicine
Mentor: Mark Hallett, M.D., Chief, Human Motor Control Section
Institute: National Institute of Neurological Disorders and Stroke (NINDS)
Research: Visual Exploration in Parkinson's Disease and Freezing of Gait



Parkinson's disease (PD) patients exhibit a number of visuospatial abnormalities. Because visual information plays a critical role in normal gait and navigation within the environment, these visual deficits may contribute to the gait problems observed in PD, including the phenomenon of freezing of gait (FOG), which is known to be induced by certain visual cues in the environment. Previous studies of visuomotor function in PD patients have been limited to computer-based tasks; for studies of visual function during dynamic tasks such as walking and turning, most research has focused on eye movement metrics and changes in gait parameters in response to specific visual cues. However, it is unclear exactly where PD patients visually explore during a dynamic task such as

walking. Our study is the first to analyze the locations PD patients visually explore while walking through a real-life environment, which may be important to understanding how visual strategies are related to gait deficits in PD. Using the SensoMotoric Instruments Eye Tracking Glasses (SMI-ETG), our study found significant differences in the visual exploration strategies between healthy individuals, PD patients without FOG, and PD patients with FOG while performing a dynamic walking task through a corridor. In particular, PD patients with FOG displayed a unique tendency to fixate significantly less on the wall at the end of the corridor that they were instructed to walk towards compared to both healthy volunteers and PD patients without FOG. In addition, patients with FOG fixated significantly more on the floor compared with the other subject groups. These differences in visual exploration could potentially play a role in the gait manifestations of PD and lead to visually-guided therapy paradigms.

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Clinical Center (CC)

Liwei Jiang

Johns Hopkins University School of Medicine
Bradford J. Wood, M.D., Director, NIH Center for Interventional
Oncology Chief, Interventional Radiology Section

National Cancer Institute (NCI)

Steven Abboud

Saint Louis University School of Medicine
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Raju Chelluri

SUNY Upstate Medical University
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Branch

Anna Chichura

Georgetown University School of Medicine
Nicholas P. Restifo, M.D., Senior Investigator, Surgery Branch

Michele Fascelli

Jefferson Medical College of Thomas Jefferson University
Peter A Pinto, M.D., Head, Prostate Cancer Division, Urologic Oncology
Branch

Richard Ho

Albert Einstein College of Medicine
Peter A. Pinto, M.D., Head, Prostate Cancer Section, Urologic Oncology
Branch

R. Spencer Kirkland, M.D.

University of Missouri School of Medicine
Kevin Camphausen, M.D., Chief, Radiation Oncology Branch
Andra Krauze, M.D., Staff Clinician Radiation Oncology Branch

Jeyan Sathia Kumar

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Multidrug Resistance Section, NCI, Deputy Director for Intramural
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Bríd Ryan, Ph.D., MPH, Stadtman Investigator, Laboratory of Human
Carcinogenesis, Center for Cancer Research

Angela Liu

University of California San Diego, School of Medicine
Nicolas Wentzensen, M.D., Ph.D., Senior Investigator, Epidemiology and
Genetics Section, Hormonal and Reproductive Epidemiology Branch

Joseph Cataquiz Rimando

Medical College of Georgia at Georgia Regents University
Daniel Fowler, M.D., Senior Investigator; Head, Cytokine Biology Section,
Experimental Transplantation and Immunology Branch

Kei Satoh

Icahn School of Medicine at Mount Sinai
Electron Kebebew, M.D., FACS, Chief, Endocrine Oncology Branch

Rebecca Ann Sorber

Indiana University School of Medicine
Udo Rudloff, M.D., Ph.D., Head, Gastrointestinal Oncology Section,
Thoracic and Gastrointestinal Oncology Branch

National Eye Institute (NEI)

Amol Sura

LSUHSC School of Medicine, New Orleans
Robert Nussenblatt, M.D., MPH, Chief, Laboratory of Immunology

Alisa Thavikulwat, M.D.

University of Rochester School of Medicine and Dentistry
Emily Chew, M.D., Deputy Director, Division of Epidemiology and Clinical
Applications
Brett Jeffrey, Ph.D., Staff Scientist, Ophthalmic Genetics and Visual
Function Branch

Jane Kim

University of California, San Diego, School of Medicine
H. Nida Sen, M.D., MHS, Director, Uveitis and Ocular Immunology
Fellowship Program
Robert B. Nussenblatt, M.D., MPH, Chief, Laboratory of Immunology

National Heart, Lung, and Blood Institute (NHLBI)

Josephine Larkin Harrington

University of Massachusetts Medical School
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Physiology

Amrita Karambelkar

Icahn School of Medicine at Mount Sinai
Richard Childs, M.D., Senior Investigator, Laboratory of Transplantation
Immunotherapy, Clinical Director, NHLBI

Taufiq Salahuddin

Wake Forest University School of Medicine
Nehal Mehta, M.D., MSCE, Lasker Clinical Research Scholar, Section of
Inflammation and Cardiometabolic Diseases

National Human Genome Research Institute (NHGRI)

Bartholt Clagett

University of Pennsylvania Perelman School of Medicine
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Research Section, Social and Behavioral Research Branch

Dino Maglic

Brody School of Medicine at East Carolina University
William Gahl, M.D., Ph.D., Senior Investigator, Medical Genetics Branch,
Clinical Director, National Human Genome Research Institute, Director,
NIH Undiagnosed Diseases Program

National Institute of Allergy and Infectious Diseases (NIAID)

Lisa Mariko Gretebeck

University of Pennsylvania School of Veterinary Medicine
Kanta Subbarao, M.D., MPH, Chief, Emerging Respiratory Viruses Section, Laboratory of Infectious Diseases

Sarah Talia Himmelfarb

George Washington University School of Medicine and Health Sciences
Yasmine Belkaid, Ph.D., Chief, Mucosal Immunology Section, Laboratory of Parasitic Diseases

Ali Jamil

New York University School of Medicine
Joshua D. Milner, M.D., Chief, Genetics and Pathogenesis of Allergy Section, Laboratory of Allergic Disease

Nikhil Jiwrajka

Johns Hopkins University School of Medicine
Thomas A. Wynn, Ph.D., Chief, Immunopathogenesis Section, Laboratory of Parasitic Diseases Scientific Director, NIH Oxford-Cambridge Scholars Program

Trisha Pasricha

Vanderbilt University School of Medicine
Thomas Wynn, Ph.D., Chief, Immunopathogenesis Section, Laboratory of Parasitic Diseases; Scientific Director, NIH Oxford-Cambridge Scholars Program

National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)

Abdulkareem Agunbiade

University of Chicago, Pritzker School of Medicine
Michael Ward, M.D., Senior Investigator, Clinical Trials and Outcomes Branch

Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)

Sara A. Armaiz Flores

University of Puerto Rico Medical Sciences Campus
Jack A. Yanovski, M.D., Ph.D., Director, Section on Growth and Obesity

Presley Garrison

Brody School of Medicine at East Carolina University
Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)

National Institute on Deafness and Other Communication Disorders (NIDCD)

Harrison Cash

Case Western Reserve University School of Medicine
Clint Allen, M.D., Clinical Collaborator, Otolaryngology Surgeon-Scientist Development Program, NIDCD
Carter Van Waes, M.D., Ph.D., Chief, Head and Neck Surgery Branch
Clinical Director, NIDCD

Jack Williams Shteamer

Emory University School of Medicine
Wade Chien, M.D., Staff Clinician, NIDCD; Assistant Professor, Department of Otolaryngology-Head & Neck Surgery, Johns Hopkins School of Medicine

National Institute of Dental and Craniofacial Research (NIDCR)

Peter W. Fung

Harvard School of Dental Medicine
Janice S. Lee, DDS, M.D., MS, Chief, Craniofacial Anomalies and Regeneration Section
Clinical Director, NIDCR

Molly Hague

University of California, San Francisco School of Dentistry
Kenneth Yamada, M.D., Ph.D., Chief, Laboratory of Cell and Developmental Biology; NIH Distinguished Investigator

National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

Alex Dinh

University of Buffalo School of Medicine and Biomedical Sciences
Jeffrey Kopp, M.D., Chief, Kidney Diseases Branch

National Institute of Mental Health (NIMH)

Cortlyn Brown

Yale University School of Medicine
Kathleen Merikangas, Ph.D., Chief, Genetic Epidemiology Research Branch

Paul Kirkpatrick Reardon

Columbia University College of Physicians and Surgeons
Armin Raznahan, M.D., Ph.D., Chief, Developmental Neurogenomics Unit, Child Psychiatry Branch

National Institute of Neurological Disorders and Stroke (NINDS)

Jason Kenneth Hsieh

Cleveland Clinic Lerner College of Medicine of Case Western Reserve University
Kareem Zaghoul, M.D., Ph.D., Staff Clinician, Functional and Restorative Neurosurgery Unit, Surgical Neurology Branch

Michael J. Feldman

University of Rochester School of Medicine and Dentistry
Prashant Chittiboina, M.D., Staff Clinician, Surgical Neurology Branch

Denise Fay Ye

Vanderbilt University School of Medicine
Mark Hallett, M.D., Chief, Human Motor Control Section

Victoria Ting-Yi Liu

University of California, San Diego, School of Medicine
Mark Hallett, M.D., Chief, Human Motor Control Section

Martin G. Piazza

Wake Forest School of Medicine
Prashant Chittiboina, M.D., Staff Clinician, Surgical Neurology Branch

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