Harold Amos Medical Faculty Development Program

Name: Kevin M. Alexander, MD

Institution: Stanford University

Mentor(s): Ronglih Liao, PhD

College: Yale University

Medical School: University of Pennsylvania

Residency: Johns Hopkins Hospital

Fellowship: Brigham and Women’s Hospital (Cardiology); Stanford Hospital (Advanced Heart Failure and Transplant Cardiology)

Title: Elucidating determinants of pathogenesis in transthyretin cardiac amyloidosis

**Background:** Transthyretin (ATTR) cardiac amyloidosis is an important, but underdiagnosed cause of heart failure. Misfolded transthyretin (TTR) protein aggregates and gradually forms amyloid fibrils that deposit in the heart, leading to biventricular hypertrophy, arrhythmias, heart failure, and death. In particular, ATTR cardiac amyloidosis is responsible for significant heart failure burden among African descendants due to a high carrier rate for the amyloidogenic V122I TTR variant. Despite the recent development of ATTR therapies, there is no cure for this deadly disease. Moreover, many patients are still only diagnosed in the later stages when treatment have minimal or no effect. A limited mechanistic understanding of ATTR amyloid formation and subsequent organ dysfunction have hampered the development of novel early diagnostics and effective therapies. Clinical observations suggest that old age and certain conditions, such as aortic stenosis, are associated with ATTR amyloid formation. We hypothesize that specific aging and hemodynamic factors promote amyloidogenesis in ATTR cardiac amyloidosis.

**Methods:** To study factors associated with ATTR amyloid formation, a novel *in vivo* mouse model was created. Adult mice were injected with an adeno-associated virus (AAV) to promote expression of human V122I TTR. To test the effect of pressure overload and shear stress on amyloidosis, V122I TTR mice underwent transverse aortic constriction (TAC) or sham surgery. To investigate whether aging promotes amyloid formation, a separate group of older mice (50-60 weeks) were injected with V122I TTR AAV. These mice underwent protein expression, histologic, and echocardiographic assessments for amyloidosis. In addition to mouse serum and heart tissue, blood and explanted heart tissue from ATTR patients are being collected via the Institutional Review Board approved Stanford Amyloid Center biobank. These samples will be used for RNA sequencing.

**Results:** The novel mouse model demonstrated reproducible, consistent expression of human V122I TTR at physiologic levels. At baseline, these animals did not show any evidence of amyloid formation. For the pressure overload experiments, the TAC animals showed significantly reduced fractional shortening and increased left ventricular hypertrophy compared to the control animals. For the aging experiments, older V122I TTR animals developed cardiac amyloid deposition confirmed by Congo Red staining and electron microscopy. The mouse hearts from these experiments (n=30) as well as explanted heart tissue from ATTR patients and controls (n=12) were sent for RNA sequencing to further evaluate the molecular changes associated with amyloid formation.

**Conclusions:** In a novel *in vivo* mouse model of V122I ATTR cardiac amyloidosis, aging and cardiac pressure overload were associated with increased amyloid formation and cardiac dysfunction. Ongoing RNA sequencing analyses will provide further insights on the specific molecular underpinnings for ATTR cardiac amyloidosis.

Harold Amos Medical Faculty Development Program

Name: LaPrincess C. Brewer, MD, MPH

Institution: Mayo Clinic College of Medicine, Division of Preventive Cardiology, Department of Cardiovascular Medicine, Rochester, MN

Mentor(s): Christi A. Patten, PhD; Lisa A. Cooper, MD, MPH

College: Howard University, Washington, DC

Medical/Dental/Nursing PhD School: George Washington University School of Medicine, Washington, DC

Residency: Johns Hopkins University/Johns Hopkins Bayview Medical Center Internal Medicine Residency Program, Baltimore, MD

Fellowship: Mayo Clinic Cardiovascular Diseases Fellowship, Mayo Clinic Preventive Cardiology Fellowship, Rochester, MN

Title of Project: *The FAITH! (Fostering African-American Improvement in Total Health) Trial: A community-based, mHealth Intervention to Improve Cardiovascular Health Among African-Americans*

**Background:** Compared to whites, African-Americans have lower prevalence of ideal cardiovascular health

(CVH) based on the American Heart Association Life’s Simple 7 (LS7). Ideal LS7 health-promoting behaviors and biological risk factors (e.g., physical activity (PA), blood pressure) are associated with improved CVH outcomes. The FAITH! (Fostering African-American Improvement in Total Health) App, a community-informed, mobile health (mHealth) intervention, previously demonstrated significant improvements in LS7 components among African-Americans, suggesting mHealth interventions may be effective in improving CVH. **Objective:** Utilizing a community-based participatory research approach, this study assessed the feasibility/preliminary efficacy of a refined FAITH! App for promoting LS7 among African-Americans in faith communities using a cluster, randomized controlled trial (RCT). We hypothesized that our behavioral theory-informed, app-based intervention would be feasible and improve CVH among AAs in faith communities from baseline to 6-months (mos) post-intervention. **Methods:** As a part of Aim 1, we conducted three focus groups on app refinement and inclusion of user-individualized/interpersonal features. Primary outcomes are refined app satisfaction/usability by the Health Information Technology Usability Evaluation Scale (Health-ITUES). For Aim 2, participants received the FAITH! App (immediate intervention) or were assigned to a delayed intervention comparator group. Data were collected via electronic surveys and health assessments at baseline and 6-mos post-intervention. Primary outcomes were change in LS7 composite score from baseline to 6-mos post-intervention and app engagement/usability. **Preliminary Results:** A total of 15 participants were recruited for the Aim 1 focus groups (mean age (SD) 56.9 (12.3) years, 87% female). There was overall high user satisfaction with the refined app and the app exceeded the usability threshold goal to proceed to use in the RCT (Health-ITUES, mean (SD) 4.4 (0.5)). Of RCT-enrolled individuals, 76 completed baseline surveys/health assessments, for a participation rate of 89% (N=34 randomized to immediate intervention, N=42 to delayed intervention [control]). At baseline, participants were predominantly female (54/76, 71%), employed (56/76, 78%) and of high cardiometabolic risk (72/76, 95% with hypertension and/or overweight/obesity) with mean LS7 scores in the poor range (6.8, SD 1.9). From baseline to 6-mos post-intervention, the mean LS7 score of the intervention group increased by 2.1 points (SD 1.9) as compared to an increase of 0.8 point (SD 1.6) of the control group (both *P*<0.0001). When adjusted for age and education level, the estimated difference of this increase between the groups was 1.23 (95% CI 0.68-1.79; *P*<0.0001). App engagement /usability was overall high (>50% completed ≥50% education modules, >75% completed weekly diet/PA tracking, Health-ITUES, mean (SD) 4.7 (0.4)). **Conclusions:** Based on preliminary findings, the refined FAITH! App appears to be an efficacious mHealth tool to promote ideal CVH among African-Americans.

Harold Amos Medical Faculty Development Program

Name: Amanda S. Bruegl

Institution: Oregon Health and Science University

Mentor(s): Cynthia Morris, PhD

College: University of Wisconsin-Madison

Medical/Dental/Nursing PhD School: University of Washington School of Medicine

Residency: University of Wisconsin

Fellowship: The University of Texas, MD Anderson Cancer Center

Title of Project: Prevalence and Distribution of high-risk HPV subtype among American Indian/Alaska Native Women living in the Pacific Northwest: A Cross-Sectional study of women living in urban and reservation-based settings

Background: Cervical cancer and its precursor lesions are due to the sexually transmitted infection human papillomavirus (HPV), and its persistence is critical for cancer development. Cervical cancer is a preventable disease through pap smear screening and HPV vaccination. Substantial disparities in both cervical cancer incidence and mortality across racial and ethnic groups persist. Our data from the Pacific Northwest show that American Indian/Alaska Native (AI/AN) women have persistently had a greater incidence and mortality rate compared to Non-Hispanic White (NHW) women.

Objective: Eliminate cervical cancer disparities faced by AI/AN women

Research Question: Is research being done in the AI/AN population?

Methods: A systematic scoping review will be performed to evaluate published literature from 1990-2020 that address cervical cancer, HPV, and cervical dysplasia in AI/AN women.

Results: Our search yielded 515 citations, 146 articles met inclusion criteria and were included in our review. The median impact factor for published articles was 2.14, (0-292). Descriptive studies represented 58.9% of all published articles followed by observational (23.2%) and interventional (11.6%). Cervical cancer screening/dysplasia was the most commonly published topic (50.6%), followed by incidence/mortality studies (23.9%), HPV (22.6%), and invasive cancer (2.7%). The Indian Health Service (IHS) regions most heavily represented were the Alaska Area and Great Plains Area. Of the 110 articles in which funding source was documented, 83.6% were federally funded, 11.8% were funded by a non-profit agency, and the remaining 4.6% were a combination of federal, non-profit, and/or private industry.

Research Question: Are AI/AN women in the PNW utilizing prevention tools (i.e. HPV vaccination and pap smear screening) to reduce their risk of cervical cancer?

Methods: Clinical encounter data between January 2010 and July 2020 from Indian Health Service (IHS), Tribal, and Urban (I/T/U) clinics in the Pacific Northwest (PNW), obtained from the IHS National Data Warehouse, were prepared and analyzed. Clinical encounters where pap smears were performed were identified using ICD-9 and ICD-10 clinical procedure codes, CPT codes, and laboratory free text notes. Cervical cancer screening rates identified patients with pap smears documented in the previous 3 years among all female clinical patients between 25 and 64 years of age with at least one clinical visit within the study period, or patients between 30 and 64 years of age with a pap screen and an HPV DNA test in the previous 5 years.

Results: In the PNW, we identified a total of 34,278 patients eligible for cervical cancer screening within the 10-year period. Aggregated up-to-date pap smear rate was 63.5% for the three-state region. Between the two time periods assessed (January 2010 – December 2014 and January 2015 – July 2020), cervical cancer screening rates increased slightly in all three states of interest with the greatest increase see in Washington state and least in Idaho. Despite these modest increases, cervical cancer screening rates remain below both the 2018 calculated national screening average of 80.5% and the Healthy People 2030 target of 84.3%.

Research Question: What barriers exist for patients receiving care at I/T/U clinics who receive abnormal cervical cancer screening results?

Methods: A survey is being developed and will be distributed at the Portland Area Indian Health Service Region medical director’s cancer update in Spring 2022 to determine contributors preventing follow-up for abnormal cervical cancer screening.

Research Question: Can telehealth and HPV self-collection be used to increase the rate of cervical cancer screening in underserved populations?

Specific Aim: Compare the efficacy and patient experience of telehealth-based, self-collected cervical cancer screening to mail-based, self-collected cervical cancer screening

Primary Endpoint: Patient preference for HPV self-collection compared to provider collection in the office

Methods: This is a prospective, randomized study of women ages 25 and older, stratified by menopausal status (defined as 12 months or greater without menses or time at which both ovaries have been surgically removed), who are eligible for cervical cancer screening. After consent, women who agree to participate and meet the entry criteria will be randomly assigned to one of the two following treatments arms: the control arm will be the mail based instructions and the intervention arm will have a telehealth visit to provide enhanced instructions.

Results: IRB is under review and we anticipate accruing patients within 4-6 weeks.

Concluding Remarks: Results from these studies will identify opportunities for interventions to eliminate cervical cancer disparities. Our preliminary data show that research is limited in the AI/AN population and that participation in pap smear screening consistently lags behind the U.S. population and national screening goals. Our survey will help identify barriers providers and women face once cervical cancer screening is abnormal and identify interventions to reduce barriers to follow-up. Finally, our pilot study will evaluate the role of telehealth and the HPV self-collection process. Results from this study can be used to expand HPV self-collection and eliminate geographic disparities with telehealth.

Harold Amos Medical Faculty Development Program

Name: Maria Isabel Carlo

Institution: Memorial Sloan Kettering Cancer Center

Mentor(s): Kenneth Offit, MD

College: Harvard College

Medical/Dental/Nursing PhD School: Harvard Medical School

Residency: Brigham and Women’s Hospital

Fellowship: Memorial Sloan Kettering Cancer Center

Title of Project: Identification of Novel Germline Variants Associated with Increased Risk of Renal Cell Carcinoma

Background: Renal cell carcinoma (RCC) is a highly heritable, and has a poor prognosis when identified in advanced stages. Most known RCC genetic syndromes are associated with distinct pathologic RCC subtypes, such as Fumarate Hydratase (FH)-deficient RCC seen in patients with germline variants in the FH gene. Despite several known genetic syndromes, the majority of familial RCC remains unexplained. To date, a key barrier to the discovery of RCC-susceptibility genes has been the lack of large cohorts of RCC patients with available genomically-profiled tumors.

Methods: To nominate and validate novel RCC-susceptibility genes, we assembled a cohort of over 1240 RCC patients who have undergone tumor and germline targeted exome sequencing for over 380 cancer-associated genes. We excluded patients with known germline variants predisposing to cancer. To enrich for patients at higher risk of hereditary syndromes, we focused on patients with more than one primary RCC or RCC and second malignancies. We analyzed the germline for predicted loss-of-function variants in cancer-associated genes. For candidate genes, we analyzed somatic data to look for loss of heterozygosity (LOH). For select patients with candidate variants, we recruited family members for co-segregation analysis.

Results: In a subgroup of RCC patients enriched for multiple primary malignancies, we identified germline and somatic predicted loss-of-function variants in the gene *KEAP1*, a known tumor suppressor gene and negative regulator of NRF2, the key activator of the antioxidant response pathway. RCCs with *KEAP1* biallelic loss had papillary or unclassified histology. In a proband with RCC and the germline *KEAP1* p.Gln217\* variant, we sequenced 4 first degree relatives, and identified that the variant co-segregated with individuals with cancer. Furthermore, we identified LOH or a second somatic *KEAP1* mutation in all 3 tumors available from the proband and family. In a pan-cancer cohort, we identified 1021 tumors with somatic *KEAP1* mutations, of which 79% had LOH in the second allele.

Conclusions: We show that the tumor suppressor *KEAP1* is a candidate RCC susceptibility gene, and that biallelic *KEAP1* loss may be a driver of tumorigenesis in RCC. RCC with *KEAP1* loss has a distinct histologic phenotype. In RCC and pan-cancer, loss of heterozygosity in the second allele appears to be a common mechanism for loss of function in tumors with *KEAP1* variants. Future work includes functional characterization of the KEAP1/NRF2 pathway in tumors with *KEAP1* loss.

**Harold Amos Medical Faculty Development Program**

Name: Jose F. Figueroa, MD, MPH

Institution: Harvard T.H. Chan School of Public Health & Brigham and Women’s Hospital

Mentor(s): Ashish Jha, MD, MPH, Dean, Brown University School of Public Health

College: University of Houston

Medical/Dental/Nursing PhD School: Harvard Medical School

Residency: Brigham and Women’s Hospital, Internal Medicine Residency

Fellowship: N/A

Title of Project: The burden of HIV/AIDS in older adults: A growing dilemma

**Abstract for Paper #1:**

**Importance:** An increasingly older HIV population raises important questions regarding how the disease may influence spending on other chronic conditions and mental health disorders.

**Objective:** To determine the degree to which HIV influences spending and utilization related to non-HIV chronic conditions and the extent to which antiretroviral therapy (ART) mitigates this relationship.

**Design:** Using a 20% sample of Medicare claims, we compared risk-adjusted spending and utilization for Medicare beneficiaries with and without an HIV diagnosis, as well as subgroups of people with HIV on ART. Spending was calculated across five major categories: direct HIV/AIDS costs, HIV-associated conditions or other infections, mental health disorders, other chronic medical conditions, and drugs/medications.

**Setting:** United States in 2016

**Participants:** Fee-for-service Medicare beneficiaries

**Exposure:** HIV diagnosis

**Main Outcomes and Measures:** Risk-adjusted spending

**Results:** Of 4,501,339 Medicare beneficiaries, 21,564 (0.5%) had an HIV diagnosis, of which 1,974 were not on ART. On average, people with HIV were younger, more likely to be Black or Hispanic, and dual-eligible. Compared to beneficiaries without HIV ($16,219), people with HIV on ART incurred 220.6% more spending ($52,004), mostly driven by ART spending ($28,854), while people with HIV not on ART incurred 95.4% more spending ($31,689). People with HIV but not on ART had higher spending related to infections (+60.3%), mental health disorders (+85.1%), and other medical conditions (+39.2%) compared to people with HIV on ART. Months on ART was associated with lower excess spending on mental and medical conditions in a dose-response manner; people with HIV treated with at least 12 months of ART incurred similar average levels of spending as people without HIV (excluding drug costs).

**Conclusions and Relevance:** An HIV diagnosis is associated with substantially higher spending among Medicare beneficiaries, mainly driven by ART spending. Overall, Medicare beneficiaries with HIV do not spend substantially more on mental health or other chronic medical conditions after adjusting for clinical complexity, except for the subset of HIV patients not on ART. These findings suggest that ART may be associated with reduced excess spending on mental health and other chronic conditions among older people. However, high ART prices need to be addressed.

Harold Amos Medical Faculty Development Program

Name: C. Rory Goodwin, MD, PhD

Institution: Duke University Medical Center

Mentor(s): John Sampson, MD, PhD, Donald McDonnell, PhD, Daniel Sciubba

College: University of Florida

Medical/Dental/Nursing PhD School: Johns Hopkins University School of Medicine

Residency: Neurosurgery, Johns Hopkins Hospital

Fellowship: Enfolded Spine Oncology and Complex Spine, Johns Hopkins Hospital

Title of Project: HRQOL, decision making, and molecular determinants of spine metastasis

Metastatic spine disease affects a significant proportion of cancer patients, and can lead to severe adverse effects, including sensory disturbances, weakness, pain, bowel/bladder incontinence and/or paralysis. The prevalence of metastatic spine disease and the functional sequelae of the disease are expected to increase due to advancements in screening, detection, and therapies. Patients with metastatic spine disease have poor prognoses and are at risk for low health-related quality of life (HRQOL). Few studies have examined patient-specific factors or interventions that influence and/or improve quality of life in this population. Furthermore, prognostication and management rely almost exclusively on clinical and imaging parameters, and thus, patient-specific priorities, preferences, and expectations are rarely elicited within the clinical decision-making landscape. Moreover, although studies have demonstrated that significant disparities exist in health outcomes and satisfaction between different sociodemographic groups (i.e. race, gender, income status, and education) in a variety of non-spine disease types, the impact of these factors on HRQOL and clinical outcomes for patients diagnosed with metastatic spine disease remains unclear. Furthermore, genomic predictors of tumor progression in spine metastasis are lacking, with tumor type providing the best indication of prognosis. During the course of fellowship period, I have completed several studies examining the full continuum of administered care for patients diagnosed with spine metastasis. Using quantitative and qualitative analyses, we examined patient and provider priorities, perceptions of interventions for metastatic spine disease, patient–provider communication, and the influence of sociodemographic factors on communication and clinical outcomes. Through analysis of the National Inpatient Sample database, we found on multivariable analysis that black patients and males were more likely to present with more severe disease, black surgical patients had increased risk of perioperative morbidity, and females and blacks were less likely to receive surgical intervention. To obtain a more granular analysis, I served as the project lead examining the influence of gender on health-related quality of life and outcomes for patients undergoing surgical intervention and/or radiotherapy and found that although female patients had better survival and better outcomes, they did not achieve the same benefit in HRQOL as males. Furthermore, we assessed the influence of interventional factors on clinical outcomes ranging from interventions to improve neurologic function, preoperative optimization, nutrition consults, radiotherapy advances, prognostic calculators, spinal alignment, and hospital-based characteristics for patients diagnosed with spine metastasis. Finally, using single cell RNA sequencing analysis of human spine metastasis clinical specimens obtained from the neurosurgical operating room, we were able to identify differences in gene expression, and cell subsets and normal versus tumor bearing vertebral bodies in the same patient. Studies will increase our understating of the determinants of spine metastasis and lead to the development of preclinical models and therapies to improve outcomes.

**AHA - Harold Amos Medical Faculty Development Program**

Name: J. Sawalla Guseh, M.D.

Current Institution: Massachusetts General Hospital, Harvard Medical School

Mentor: Anthony Rosenzweig, M.D.

College: Harvard College (Cambridge, MA)

Medical School: Harvard Medical School (Boston, MA)

Fellowship: Cardiovascular Diseases, Massachusetts General Hospital

Postdoctoral Fellowship: Basic Cardiovascular Disease Biology (Rosenzweig Lab)

Title: Shrinking Enlarged Hearts: Translation of Cardiac Regression Pathways from Burmese Python to Human

**Background**: Over 6 million Americans have heart failure (HF) and prevalence will increase 46% by 2030. Importantly, **cardiac hypertrophy** remains the dominant risk factor for heart failure (HF) that predicts stroke, arrhythmia, ischemic heart disease, and sudden cardiac death. A key clinical insight remains that cardiac hypertrophy commonly and silently *precedes* clinical HF as a subclinical intermediate phenotype and therefore **is a key cardiovascular phenotype.** Longitudinal clinical studies demonstrate that even a partial decrease in heart mass or *regression* is independently associated with improved cardiovascular outcomes. Accordingly, regression strategies to understand and reverse pathological hypertrophy are enticing as therapeutic targets for HF and CVD prevention.

**Objective**: Our goal is to use a range of model systems, including the extreme regression physiology of the Burmese Python, to develop a greater transcriptional understanding of the pathways that control cardiac regression. We aim to use this understanding to develop therapeutic candidates that might facilitate beneficial human regression to prevent and treat heart failure and hypertrophic heart diseases.

**Methods**: We used RNA-Seq, multiple models of myocardial regression (fasted animals, experiment heart failure, TAC-Debanding, pythonic regression) and principal component analysis to identify a common transcriptional program associated with heterogenous models of myocardial regression. Second, we used small RNA-Seq in the Burmese Python to identify microRNA associated with growth and regression. Using statistical significance, fold change, and species conservation we designed chemical miRNA mimics to examine the functional their functional effects on *in vitro* cardiomyocytes.

**Results**: First, PCA analysis using RNASeq data from multiple growth and regression states reveals that growth and regression can be captured coherently along the first principal component (PC1). Second, the PC1’s loadings reveal known and novel genes associated with myocardial growth (*ACTA1*) and myocardial regression (*FBXO32*). ~31% of the transcriptional variance is explained by PC1 supporting the hypothesis that myocardial regression, similar to pathological and physiological growth, likely reflects heterogenous stages. Third, deep sequencing at various time points in the Burmese Python revealed ~50 dynamically regulated (p < 0.05) miRNA associated with growth and regression. One species conserved miRNA (in python, mouse, rat, human) showed isolated expression in the regression phase of the feeding cycle and was undetectable in other states raising interest that it may act as a “biological switch”. Chemical mimics of this miRNA consistently abrogate a hypertrophic “pathological” transcriptional program in cultured primary cardiomyocytes and reduce cardiomyocyte size by ~12% (p = 0.0038).

**Conclusions**: Like cardiac hypertrophy, profiling of myocardial regression reveals an underlying transcriptional program that marks a heterogenous regression state. More work is needed to better understand the variation observed in myocardial regression. Secondly, deep sequencing of the Burmese Python reveals a promising miRNA that abrogates a pathological growth program and reduces cardiomyocyte size. This miR is known to be present in human hearts, circulates in plasma, and is associated with clinical heart failure. We are examining its ability to regress hypertrophied TAC heart. Finally, given it circulates in human plasma, we are assessing its ability to predict myocardial regression in a human aortic stenosis population undergoing transcatheter aortic valve replacement (TAVR) where heterogeneity of regression is observed after the procedure.

Harold Amos Medical Faculty Development Program

Name: Rasheeda K. Hall MD, MBA, MHS

Institution: Duke University School of Medicine

Mentor(s): Cathleen Colón-Emeric MD, MHS; Julia Scialla MD, MHS

College: Vanderbilt University

Medical/Dental/Nursing PhD School: Vanderbilt University School of Medicine

Residency: Internal Medicine, Duke Department of Medicine

Fellowship: Nephrology, Duke Department of Medicine

Title of Project: Establishing Evidence to Manage Geriatric Syndromes in Dialysis Patients

Problem: Compared to older adults without kidney disease, older dialysis patients are twice as likely to fall, develop severe cognitive impairment, and become hospitalized. These adverse outcomes are associated with potentially inappropriate medications (PIMs). PIMs are medications such as benzodiazepines and anticholinergics that should be avoided in older adults because their risks usually outweigh their benefits. Older dialysis patients are more susceptible to adverse effects of PIMs because of altered medication clearance, blood pressure fluctuations during dialysis, and comorbid cerebrovascular disease. Given this susceptibility, reduction of PIMs is a logical first step towards improving quality of care for these vulnerable patients.

Objective: The objective is to develop an evidence-based strategy to reduce PIM use in older dialysis patients. Dr. Hall’s concurrent NIH grant examines prescribing patterns of specific PIMs, risk of hospitalization associated with those PIMs, stakeholder input from patients aged ≥65 years, and a subsequent pilot deprescribing intervention. To complement that grant, the AMFDP award supports specific studies in young, frail adults to uncover the following evidence: 1) prescribing patterns of specific PIMs and their associated risk in frailty incidence (among non-frail) and/or functional decline, and 2) insight into elements of a deprescribing intervention from younger, frail dialysis patients.

Approach: We use the United States Renal Data System to conduct epidemiologic studies on PIM prescribing patterns and risk of harm. PIMs of interest include alpha blockers, central alpha agonist, opioids, muscle relaxants, sedatives, and anticholinergics. To inform pilot design, we conducted qualitative study with 53 clinicians (dialysis, primary care, and pharmacists) and 21 patients/caregivers (11 were aged 55-64 years).

Findings: Using a cohort of older adults new to dialysis (2013-2014), we found that continuation of an antihypertensive PIM after dialysis initiation was not associated with increased hospitalization or mortality risk. *BMC Nephrol* 22, 232 (2021). Key themes to inform pilot design were: 1) clinicians have limited communication, time, and knowledge, and 2) patients trust primary clinician opinion, prefer fewer medications, but also value symptom relief.

Next steps: 1) Pilot deprescribing model of care at local dialysis clinics; 2) Conduct pharmacoepidemiologic study to identify extent of association of PIMs with onset of frailty and functional decline in adults receiving dialysis.

Harold Amos Medical Faculty Development Program

Name: Tamia Harris-Tryon, MD, PhD

Institution: UT Southwestern Medical Center

Mentor(s): Lora Hooper, PhD

College: Haverford College

Medical/Dental/Nursing PhD School: Johns Hopkins School of Medicine

Residency: Johns Hopkins Hospital

Fellowship: UT Southwestern Medical Center

Title of Project: The Function of Small Proline Rich Proteins in Cutaneous Host Defense

Human skin functions as a physical barrier, preventing the entry of foreign pathogens while also accommodating a myriad of commensal microorganisms. A key contributor to the skin landscape is the sebaceous gland. Mice devoid of sebocytes are prone to skin infection, yet our understanding of how sebocytes function in host defense is incomplete. Here we show that the small proline-rich proteins, SPRR1 and SPRR2 are bactericidal in skin. SPRR1B and SPPR2A were induced in human sebocytes by exposure to the bacterial cell wall component lipopolysaccharide (LPS). Further, LPS injected into mouse skin triggered the expression of the mouse SPRR orthologous genes, *Sprr1a* and *Sprr2a*, through stimulation of MYD88. Both mouse and human SPRR proteins displayed potent bactericidal activity against MRSA (methicillin-resistant *Staphylococcus aureus*), *Pseudomonas aeruginosa* and skin commensals. Thus, *Sprr1a-/-;Sprr2a-/-* mice are more susceptible to MRSA and *Pseudomonas aeruginosa* skin infection. Lastly, mechanistic studies demonstrate that SPRR proteins exert their bactericidal activity through binding and disruption of the bacterial membrane. Taken together, these findings provide insight into the regulation and antimicrobial function of SPRR proteins in skin and how the skin defends the host against systemic infection.

**Harold Amos Medical Faculty Development Program**

Name: Gabriela Hobbs

Institution: Massachusetts General Hospital, Harvard Medical School

Mentor (s): Ann Mullally, Daniel Deangelo

Medical School: Mount Sinai Medical School

Residency: Brigham and Woman’s Hospital

Fellowship: Memorial Sloan Kettering Cancer Center

**Title of Project:** Improving allogeneic stem cell transplant outcomes in patients with myelofibrosis

**Background:**

Myelofibrosis (MF) is a lethal hematological malignancy associated with somatic mutations in JAK2, CALR or MPL. Ruxolitinib is the first JAK1/2 inhibitor approved for treatment of MF. Ruxolitinib does not prevent disease progression and thus, allogeneic hematopoietic cell transplantation (HCT) remains the only curative treatment. Ruxolitinib discontinuation, in preparation for HCT is challenging as patients experience return of symptoms/splenomegaly. Therefore, ruxolitinib is often continued during and after HCT in an off-label fashion, yet little is known about the safety of this approach.

Outcomes for patients with MF undergoing HCT have historically been poor, with overall survival (OS) estimates of 40% at 2 years. The leading causes of morbidity and mortality after HCT include infection, relapse and graft versus host disease (GVHD), which are not unique to MF. However, prolonged cytopenias after HCT are a particular challenge for MF patients due to poor graft function related to splenomegaly, which is common in MF, and underlying fibrosis that takes months to reverse.

The primary goal of this project is to investigate the safety and efficacy of ruxolitinib administered during transplantation. The exploratory aims of this research are to investigate the role of genetic testing after transplant to predict outcomes of patietns with MF undergoing HCT.

**Methods:**

I am conducting a phase II, multi-center, investigator-initiated trial investigating ruxolitinib given pre-, during- and post-HCT for patients with primary or secondary MF(NCT03427866). The accrual goal is 48 patients with 1-year GVHD free and relapse free survival (GRFS) as the primary endpoint. Secondary endpoints include overall and progression free survival, engraftment and incidence of acute and chronic GVHD, respectively. Patients will remain on ruxolitinib 5 mg BID until blood counts engraft at which point they may escalate to 10 mg BID.

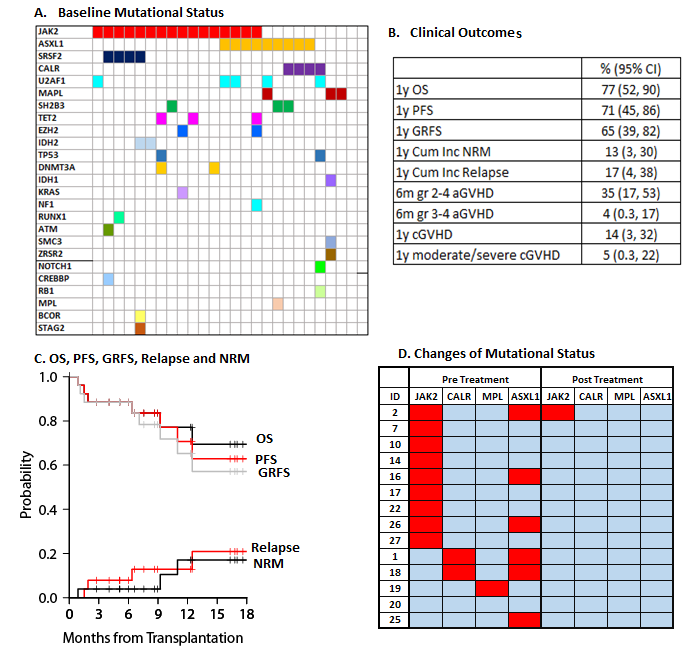
Patient samples are collected before and at day 100 after HCT and an extended next generation sequencing (NGS) panel is utilized to investigate changes in genetic mutations with HCT. In addition, single-cell profiling at the same timepoints will be done to evaluate changes in the bone marrow composition with HCT.

**Results:** Between September 2018- and January 2021 26 patients have enrolled. Median age was 66 (range 46, 75) and 65% were male. 88% had 8/8 matched related grafts, and 92% had intermediate-2 or high risk disease by Dynamic International Prognostic Scoring System (DIPSS) at the time of HCT. At HCT, 58% had JAK2, 12% CALR, 12% MPL, and 35% ASXL1 mutations (Figure A). Ruxolitinib was well tolerated. The most common grade 3/4 hematologic adverse events (AE) were anemia (n=4), thrombocytopenia (n=3). There were few grade 3/4 non hematologic AEs that included infection (n=2) and hypertriglyceridemia (n=1). Median time to neutrophil engraftment was 15 days (range 11-38) after HCT. All but one patient achieved successful neutrophil engraftment. Clinical outcomes are summarized in Figure B. With median follow-up among survivors of 12 months (range 3, 24), 1-yr GRFS was 65%. OS, PFS, and cumulative incidence of NRM and relapse were 77%, 71%, 13% and 17%, respectively (Figure C). Cumulative incidence of grade II-IV and grade III-IV acute GVHD was 35% and 4%, respectively. There was no grade IV acute GVHD and only one case of grade III acute GVHD. Cumulative incidence of all chronic GVHD and moderate-severe chronic GVHD was 14% and 5%, respectively. There was no severe chronic GVHD and only one patient developed moderate chronic GVHD.

In terms of genetic testing; results are available for 14 patients with paired samples, including 6 with ASXL1 mutations. All but one patient, who remains in remission at last follow up, no longer had mutations detected by NGS at day 100 (Figure D). No patient samples have been successfully processed for single cell profiling thus far as the protocol was recently amended to allow sample collection for this purpose and the trial was on hold but due to reopen at the end of 2021.

**Discussion:**

Allogeneic HCT is the only curative treatment for patients with MF. Therapies that improve the morbidity and mortality associated with HCT are critical. Thus far, the results of the multicenter study demonstrate safety of ruxolitinib administration pre, during and post-HCT with very favorable engraftment rates and no unexpected toxicities of ruxolitinib use. In addition, the results of PFS, OS and GRFS are superior compared to historical reports. Incidence of severe acute and chronic GVHD are thus far minimal indicating excellent GVHD control with prophylactic ruxolitinib use. In terms of changes in mutational profile, most patients have lost their pre-HCT mutations. In terms of remission assessment and exploratory analysis, most patients appear to have a significant change in mutational status with HCT. One patient that had a detectable JAK2 mutation at day 100 remains in remission. Therefore, further investigation will include single gene PCR to more accurately detect the presence of mutations after transplant. At the completion of the trial, we will correlate 1 year survival and progression data with mutational profiles at day 100 after transplant.



Harold Amos Medical Faculty Development Program

Name: Autumn S Ivy. MD PhD

Institution: UC-Irvine School of Medicine and Children’s Hospital Orange County

Mentor(s): Dr. Tallie Z. Baram, Dr. Marcelo Wood, Dr. Dan Cooper

College: Cal State Los Angeles

Medical/Dental/Nursing PhD School: UC Irvine School of Medicine

Residency: Pediatrics, Lucile Packard Childrens Hospital, Stanford University

Fellowship: Child Neurology, Stanford University

**Title: Neural Epigenetic Mechanisms of Early Life Adversity and Exercise Intervention**

ABSTRACT: The mammalian brain undergoes protracted postnatal development and is particularly sensitive to the enduring effects of early-life experiences. Understanding how these experiences can influence the trajectory of brain development and function throughout the lifespan could lead to the development of novel strategies to ameliorate deficits in cognitive function, both in childhood and with aging. Molecular signatures of early-life experiences can be discovered through new tools in next-generation sequencing that allow for the coupling of transcriptomic and epigenomic data from within individual neurons or neuronal populations. This approach can reveal novel epigenetic targets for intervention as well as identify temporal specificity for targeting critical periods of development. Our lab focuses on the cognitive impact of early-life exercise in the setting of typical neurodevelopment, as well as considering exercise mechanisms as interventional strategies in mouse models of early-life adversity. We recently discovered that exercised juvenile rodents exhibited improvements in long-term memory, LTP, and basal synaptic physiology that lasted into adolescence (Ivy et al., Scientific Reports 2020). We have now developed a transgenic mouse line for simultaneous analysis of both the chromatin landscape and transcriptional profile of isolated neurons in a brain subregion-specific manner. We cross NuTRAP mice (Roh, *et. al.* 2017), with an EMX-Cre line; the *Emx* gene being primarily expressed in excitatory neurons. I will present data from simultaneous CUT&RUN (Skene, *et. al.* 2018)-seq and RNA-seq experiments on hippocampal neurons isolated using exercised- and non-exercised Emx-NuTRAP mice. This innovative approach is, to our knowledge, the first attempt to use NuTRAP technology in neurons. Finally, this approach will be applied to a mouse model of early-life adversity to identify molecular targets that can be changed by an exercise experience, with the goal to buffer the consequences of the adversity on cognitive function. These experiments will give us new insight into the effects of early-life experiences on the genomic and epigenomic mechanisms underlying memory performance.

Harold Amos Medical Faculty Development Program

Name: Joshua J. Joseph, MD, MPH, FAHA

Institution: The Ohio State University

Mentor(s): Sherita Golden, MD, MHS, FAHA, Willa A. Hsueh, MD

College: Morehouse College

Medical/Dental/Nursing PhD School: Boston University School of Medicine

Residency: Yale Traditional Internal Medicine Program

Fellowship: Johns Hopkins University School of Medicine

Title of Project: Association of Serum Aldosterone and Plasma Renin Activity With Ambulatory Blood Pressure in African American – The Jackson Heart Study

BACKGROUND: The renin-angiotensin-aldosterone system (RAAS) is an important driver of blood pressure (BP), but the association of the RAAS with ambulatory BP (ABP) and ABP monitoring phenotypes among African Americans has not been assessed.

METHODS: ABP and ABP monitoring phenotypes were assessed in 912 Jackson Heart Study participants with aldosterone and plasma renin activity (PRA). Multivariable linear and logistic regression analyses were used to analyze the association of aldosterone and PRA with clinic, awake, and asleep systolic BP and diastolic BP (DBP) and ABP monitoring phenotypes, adjusting for important confounders.

RESULTS: The mean age of participants was 59Å}11 years and 69% were female. In fully adjusted models, lower log-PRA was associated with higher clinic, awake, and asleep systolic BP and DBP (all P<0.05). A higher log-aldosterone was associated with higher clinic, awake, and asleep DBP (all P<0.05). A 1-unit higher log-PRA was associated with lower odds of daytime hypertension (odds ratio [OR] 0.59 [95% CI, 0.49–0.71]), nocturnal hypertension (OR, 0.68 [95% CI,0.58–0.79]), daytime and nocturnal hypertension (OR, 0.59 [95% CI, 0.48–0.71]), sustained hypertension (OR, 0.52 [95% CI, 0.39–0.70]), and masked hypertension (OR 0.75 [95% CI, 0.62–0.90]). A 1-unit higher log-aldosterone was associated with higher odds of nocturnal hypertension (OR, 1.38 [95% CI, 1.05–1.81]). Neither PRA nor aldosterone was associated with percent dipping, non-dipping BP pattern, or white-coat hypertension. Patterns for aldosterone:renin ratio were similar to patterns for PRA.

CONCLUSIONS: Suppressed renin activity and higher aldosterone:renin ratios were associated with higher systolic BP and DBP in the office and during the awake and asleep periods as evidenced by ABP monitoring. Higher aldosterone levels were associated with higher DBP, but not systolic BP, in the clinic and during the awake and asleep periods. Further clinical investigation of novel and approved medications that target low renin physiology such as epithelial sodium channel inhibitors and mineralocorticoid receptor antagonists may be paramount in improving hypertension control in African Americans.

Harold Amos Medical Faculty Development Program

**Name:** Tamorah Lewis MD, PhD

**Institution:** Children’s Mercy Hospital, University of Missouri Kansas City School of Medicine

**Mentor(s):** J Steven Leeder and Jeff Reese

**College**: Boston College

**Medical/Dental/Nursing PhD School**: The Johns Hopkins University School of Medicine

**Residency**: Pediatrics, The Johns Hopkins University School of Medicine

**Fellowship**: Neonatal / Perinatal Medicine & Clinical Pharmacology, The Johns Hopkins University School of Medicine

**Title of Project:** The effects of age and genetics on exposure to nonsteroidal anti-inflammatory drugs in premature infants

**Background** With traditional mg/kg dosing of indomethacin in preterm infants, systemic plasma drug exposures range 14-fold. Given that indomethacin therapy in the NICU is associated with unpredictable clinical efficacy and toxicity, it is imperative to refine our understanding of the variables which influence the dose-exposure relationship. We aim to develop a pharmacokinetic model in preterm infants which will be used for dose-individualization in prospective studies.

**Methods** A single center prospective cohort study enrolling all infants less than 32 weeks gestational age at birth treated with indomethacin within the first month of life. After consent obtained, infants had plasma, dried blood spot and urine samples collected for quantification of indomethacin and metabolites via mass spectrometry / high-performance liquid chromatography (MS/HPLC). In addition, we collected samples for DNA isolation and genotyping. Demographic and clinical drug response was recorded. Pharmacokinetic modelling was performed in NONMEM.

**Results** 53 preterm infants had full data available for analysis. The data from the first 38 infants were used for model development and the remaining 15 were reserved for model validation. Most infants received the first dose of indomethacin at less than 24 hours after birth (range 0.01 days to 18.2 days). The median weight at treatment was 779 grams (range 445-2630 grams). The median gestational age at time of treatment was 26 weeks (range 22 weeks – 34 weeks). Based on a population pharmacokinetic model, the population estimate volume of distribution for indomethacin was 0.47 L/kg, the renal clearance 0.017 ml/kg/hr and the hepatic clearance 0.49 ml/kg/hr. Postnatal age and CYP2C9 genotype were significant covariates for clearance in the final PK model. The PK model was able to accurately capture plasma and urine drug concentrations in the validation cohort.

**Next Steps** Weight-based dosing does not sufficiently account for extremely under-developed clearance and rapid maturation of drug disposition in the first weeks after birth. This population pharmacokinetic model, also accounting for the effect of age and genotype, will be used to build a dose-individualization tool. This tool has the goal to standardize plasma drug exposure by giving each neonate a unique dose. This dose-individualization approach will be prospectively compared to standard weight-based dosing, with the hypothesis that a precision therapeutics approach will lead to improved efficacy and decreased toxicity.

Harold Amos Medical Faculty Development Program

Name: Carlos Murga-Zamalloa

Institution: University of Illinois at Chicago

Mentor(s): Megan Lim and Ryan Wilcox

College:

Medical/Dental/Nursing PhD School: M.D. San Martin de Porres University (Peru)

Residency: University of Michigan

Fellowship: University of Michigan

Title of Project: Delineating the role of Wiskott-Aldrich syndrome protein in T-cell

lymphoma progression

Peripheral T-cell lymphomas account for approximately 6% to 10% of the total number non-Hodgkin lymphoma cases per year. These group of neoplasms are characterized by an aggressive behavior; approximately 75% of the patients will relapse after initial therapies, and the overall survival after relapse is 6 months. The engagement of the T-cell receptor molecule in neoplastic T-cell lymphoma cells promote the survival and chemotherapy resistance in a proportion of peripheral T-cell lymphomas that are characterized by expression of the transcription factor GATA-3. Previous evidence has demonstrated that the actin regulatory protein Wiskott-Aldrich syndrome protein (WASp) can serve as an adaptor protein to convey T-cell receptor-dependent signals in healthy T-cell lymphocytes. Therefore, we decided to evaluate if WASp can have a role downstream of the oncogenic T-cell receptor activation in aggressive T-cell lymphomas. For that, we evaluated the expression of WASp in the most common peripheral T-cell lymphoma subtype in the United States; peripheral T-cell lymphoma non-otherwise specific (PTCL-NOS). Our findings demonstrate that WASp is expressed in approximately 60% of PTCL-NOS cases (n = 87), and this is associated with worse clinical outcomes. Expression of the phosphorylated Y290-WASp isoform positively correlates with T-cell lymphomas within the GATA-3 group, which shows a GATA-3 gene expression signature (p < 0.005). Loss-of-function approaches with shRNA mediated knock-down of WASp in primary T-cell lymphoma samples, and cell lines demonstrated that expression of WASp is required for GATA-3 expression downstream of the T-cell receptor engagement. To further evaluate the oncogenic role of WASp downstream of T-cell receptor engagement, we evaluated the proliferation and chemotherapy sensitivity after knock-down of WASp expression. Our findings demonstrated that downregulation of WASp is associated with decreased proliferation and chemotherapy-sensitivity in T-cell lymphoma primary cells and cell lines upon engagement of the T-cell receptor. Our findings also demonstrated that the complex of Src family of kinases can regulate the phosphorylation of Y290-WASp. Inhibition of Src kinases with selective inhibitory compounds prevented T-cell receptor mediated signaling, including GATA-3 upregulation in T-cell lymphomas. Importantly, Src kinase inhibition was associated with decreased proliferation of T-cell lymphoma lines and primary samples. The overall findings suggests that WASp expression is critical during the activation of oncogenic signaling cascades downstream of T-cell receptor engagement in T-cell lymphomas, and that selective inhibition of Src signaling proteins in T-cell lymphomas may constitute a novel therapeutic option.

**Harold Amos Medical Faculty Development Program**

**Name:** Enihomo Obadan-Udoh, DDS, MPH, Dr.Med.Sc.

**Institution:** University of California San Francisco

**Mentor(s):** Elizabeth Mertz, MA, PhD, Muhammad Walji, MS, PhD

**College:** N/A

**Medical/Dental/Nursing PhD School:** University of Lagos, Nigeria

**Residency:** Harvard School of Dental Medicine

**Fellowship:** N/A

**Title of Project:** Understanding Diagnostic Failures in Dentistry: A Three-pronged Approach

**Abstract:**

**Project Summary:** In the United States (US), an estimated 5% of adults experience a diagnostic error in the outpatient setting. In a literature review of 182 case reports, one-quarter (23%) of reported dental adverse events (AEs) were associated with mis-, missed diagnoses, or delayed diagnoses. Dentists need to be able to accurately assess their current levels of diagnostic performance, understand the factors that contribute to dental diagnostic failures, and develop innovative strategies to improve the quality of diagnoses. This proposal seeks to expand the depth and breadth of knowledge about factors that underlie diagnostic failures within the dental care setting. Specifically, we will use a mixed-methods approach to 1) Develop a Repository (Collection) of Dental Diagnostic Failures, 2) Identify Common Contributory Factors to Dental Diagnostic Failures from the Providers’ Perspective, and 3) Evaluate Patient Experiences of Dental Diagnostic Failures and Their Sequelae.

**Progress Report:**

**Aim 1:** We conducted a scoping review of the biomedical literature. Our initial search yielded 2693 publications (Final search date: May 5, 2021). After the removal of duplicates, we had 1857 publications. We are currently screening the titles and abstracts to identify all potentially relevant publications. The next phase of the project will be to conduct a full text screening, data extraction, and a qualitative synthesis.

**Aim 2:** A 20-item survey was distributed to a national sample of dentists (n=40,000) over a five-week study period (May 24-June 25, 2021). We received 334 completed responses and 293 partial responses (627 total responses; 1.6% response rate). Preliminary descriptive statistics revealed that majority of participants were aged 65-74 years (12.9%), Male (68.3%), Non-Hispanic White (74.3%), general dentists (69.5%), located in California (15%), New York (7.8%), and Texas (6.6%), and had >25 years in practice (50.3%). About two-thirds practiced in a solo/small private practice (2-9 dentists) and saw an average of 40+ patients per week. 45.5% had not received any formal training on diagnostic errors in dentistry. The most error-prone dental diagnoses were acute and chronic sinusitis, diseases of pulp, periapical tissues, and other disorders of teeth and supporting structures, and head and neck cancers/ neoplasms. Failures/Delays/Errors with ordering, performing, processing, interpreting, or following up on needed tests, radiographs, or pathology results (33.5%) was the most frequent failure point within the diagnostic process. The most frequently reported contributory factors included: Incomplete history taking or examination (39.1%), Poor communication (34.8%), and Overconfidence about one’s own diagnostic ability (35.7%). While most dentists reported observing diagnostic errors made by other dentists every week, they reported making diagnostic errors themselves only quarterly. 5.5% said they had never made a diagnostic error. Increased training on diagnostic reasoning (21.9%) and improved access to or availability of specialists (8.2%) were the most recommended interventions.

**Aim 3:** We have developed the content for the Facebook Recruitment Ad, a patient screening survey in Qualtrics, and a telephone interview guide. We will begin participant recruitment in November 2021.

Harold Amos Medical Faculty Development Program

Name: Oluwatoyosi A. Onwuemene

Institution: Duke University School of Medicine

Mentor(s): Dr. Edward Wong

College: Wesleyan College

Medical/Dental/Nursing PhD School: Duke University School of Medicine

Residency: Duke University Medical Center

Fellowship: McGaw Medical Center of Northwestern University

Title of Project: Apheresis Specific Outcomes to Facilitate a Clinical Trial in Heparin-Induced Thrombocytopenia

**Background**: Autoimmune disorders like heparin-induced thrombocytopenia (HIT) cause death and disability in over 24 million Americans; but > 50% of these disorders can be treated with therapeutic plasma exchange (TPE). Despite TPE’s effectiveness, however, TPE is associated with increased morbidity, including higher hospital length of stay (LOS), increased number of ICU days, and, in some patient populations, increased mortality. Improving TPE-specific outcomes has proven difficult, largely due to the fact that single institutional studies for any one apheresis-treated disorder are limited by small sample sizes and disease-specific outcomes. However, apheresis-specific outcomes are needed to determine TPE’s impact and thus improve outcomes. Our hypothesis is that apheresis-specific outcome measures can be developed and applied to a multicenter study of HIT. To test this hypothesis, we will use multivariable modeling to determine the impact of TPE on hemostasis and thrombosis outcomes in patients with HIT.

**Interim Methods**: In a cross-sectional analysis of the National Inpatient Sample (2010-2014) and a retrospective single institution study (2012-2017), we identified patients with a diagnosis of HIT treated with or without TPE. Patient characteristics included age, sex, race, diagnoses, procedures, and comorbidities. Comorbidity burden per hospitalization was quantified using the Elixhauser comorbidity index, a validated numeric score that describes the comorbidity index. The primary outcome was in-hospital mortality, defined as death at any time during the hospitalization. Secondary outcomes included major bleeding, thrombotic complications, LOS, and hospital charges. The thrombotic complications variable was comprised of venous thromboembolism, pulmonary embolism, arterial thrombosis, amputation, and thrombotic stroke (cerebral artery thrombosis). Major bleeding was comprised of intracranial, gastrointestinal, genitourinary, hemarthrosis, pulmonary, and other bleeding

**Interim Results**: Our single institution study identified 24 patients who received TPE for HIT. Thromboembolic complications occurred in three patients (12.5%) within 7 days of TPE. Thirty-day mortality rate was 12.5% and were adjudicated to be unrelated to the TPE procedure. Our cross-sectional analysis identified 22,165 cases with HIT, among which 90 (0.4%) received TPE, corresponding to national weighted estimates of 106,435 HIT and 439 TPE-treated HIT cases. TPE was not associated with decreased in-hospital mortality (OR = 1.72; 95%CI: 0.93-3.17, p = 0.085 but was associated with a higher likelihood of major bleeding (OR = 2.35; 95%CI: 1.40-3.68, P = 0.0009) TPE was also associated with higher hospital LOS (20.5 vs 10 days, p < 0.0001) and charges (USD 211181 vs USD 81654, P < 0.0001).

**Interim Conclusions**: TPE treatment in HIT is associated with increased bleeding complications. A multi-center retrospective and prospective study is currently underway to further study bleeding outcomes of TPE-treated patients with HIT.

Harold Amos Medical Faculty Development Program

Name: Hasina Outtz Reed, M.D. Ph.D.

Institution: Weill Cornelle Medicine

Mentor(s): Augustine Choi, M.D.

College: Princeton University

Medical/Dental/Nursing PhD School: Columbia University

Residency: University of Pennsylvania

Fellowship: University of Pennsylvania

Title of Project: “Lymphatic vascular dysfunction in emphysema: uncovering the missing link”

Rationale: The lymphatic vasculature is critical for lung function, but whether there are defects in lymphatic function in lung disease is understudies. In mice, lymphatic dysfunction alone is sufficient to cause lung injury with many of the hallmarks of human emphysema. Whether there are changes in lymphatic function in cigarette smoke (CS)-induced emphysema is unknown.

Objective: We investigated whether lung lymphatic function is altered in the pathogenesis of CS-induced emphysema.

Methods: Lung lymphatics in patients with emphysema were analyzed using immunohistochemistry and compared to lung lymphatics in control smokers. Using a mouse model of CS-induced emphysema, we analyzed lung lymphatics using immunohistochemistry, drainage and cell trafficking assays, and confocal microscopy. Thoracic lymph from CS-exposed mice was harvested for proteomic analysis and compared to lymph from control mice. The effect of CS on lymphatic endothelial cell permeability was assessed using *in vitro* transport assays.

Measurements and Main Results: Analysis of human lung tissue revealed significant lung lymphatic thrombosis in patients with emphysema compared to control smokers, and that this increased with disease severity. In a mouse model, CS exposure led to lung lymphatic thrombosis, decreased lymphatic drainage, and impaired leukocyte trafficking. Analysis of thoracic lymph confirmed enrichment of inflammatory and coagulation pathways in the lymphatics of CS-exposed mice compared to control mice. *In vitro* assays demonstrated a direct effect of CS on lymphatic endothelial cell integrity.

Conclusions: CS exposure causes lung lymphatic dysfunction with thrombosis, impaired leukocyte trafficking, and changes in the composition of thoracic lymph. In patients with emphysema, lymphatic thrombosis is seen in severe disease.

**Harold Amos Medical Faculty Development Program**

**Name:** Fatima Rodriguez, MD, MPH

**Current Institution:** Stanford University School of Medicine

**Mentor(s):** Paul Heidenreich MD, MS and Latha Palaniappan, MD, MS

**College:** University of Pennsylvania

**Medical School:** Harvard Medical School/Harvard School of Public Health

**Residency:** Internal Medicine, Brigham and Women’s Hospital, Harvard Medical School

**Fellowship:** Cardiovascular Disease, Stanford University

**Title of Project**: *Cardiovascular Disease Risk Prediction and Prevention in Diverse Populations*

**Background:** There is little data on optimal cardiovascular disease prevention strategies for Hispanic and Asian populations, the two largest and most rapidly growing minority groups in the U.S.

**Objective:** To improve risk prediction models and prevention approaches for cardiovascular disease in diverse groups, including understudied disaggregated Hispanic and Asian populations.

**Methods:** This study leveraged data from an existing EHR-based cohort in Northern California that is uniquely enriched with disaggregated Hispanic and Asian patients. First, the performance of the Pooled Cohort Equations (PCE) was tested in this cohort (measured by calibration and discrimination) for disaggregated race/ethnic subgroups. Second, an ensemble of supervised machine learning (ML) models (gradient boosting machine, random forest, Lasso penalty, and extreme gradient boosting) were developed for atherosclerotic cardiovascular disease (ASCVD) risk prediction. Finally, weighted-K-nearest-neighbor (wKNN) regression models were used to identify historical EHR patients to model statin treatment decisions for primary prevention associated with the greatest percentage reduction in low density lipoprotein cholesterol (LDL-C) at 1 year of follow-up.

**Results:** The PCE overestimated ASCVD risk across this contemporary cohort by 20-60%, with heterogeneity by racial/ethnic subgroups. The predicted-to-observed ratio of ASCVD events ranged from 1.1 for Puerto Rican patients to 1.9 for Chinese patients. The discrimination also varied by subgroup and recalibration did not significantly improve the PCE’s performance. In the second analysis, the top performing ML model (gradient boosted machine) had improved performance (AUC 0.84, 95% Confidence Interval [CI]: 0.83-0.85) as compared with the PCE (AUC 0.78, 95% CI: 0.76-0.79). Incorporating additional structured EHR variables beyond traditional risk factors did not improve the model’s performance. Models performed well for Asian and Hispanic patients. An algorithm that used historical patient data was able to identify the statin treatment decision associated with the greatest LDL-C reduction. Based on the algorithm, nearly half (48%) of patients were recommended low- or moderate-intensity statins for maximum LDL-C reduction.

**Conclusions**: There is significant heterogeneity in disaggregated risk assessment by racial/ethnic subgroup. ML models allow for risk discrimination in a larger group of patients who did not meet eligibility for PCE estimates, as is common in clinical practice. EHR-based models may be useful to guide treatment decisions in areas of clinical uncertainty among diverse patients. A data-driven personalized statin recommendation approach may inform shared decision making in areas of uncertainty and highlight statin efficacy-effectiveness gaps in real-world settings.

Harold Amos Medical Faculty Development Program

Name: Ahmara G. Ross, MD PhD

Institution: University of Pennsylvania, Scheie Eye Institute

Mentor(s): Kenneth Shindler, MD, PhD and Jean Bennett, MD, PHD

College: Bryn Mawr College

Medical/Dental/Nursing PhD School: Jefferson Medical School/ Thomas Jefferson University

Residency: University of Pittsburgh

Fellowship: University of Pennsylvania, Scheie Eye Institute Neuro-ophthalmology (2016-2017) and Glaucoma (2017 to 2018)

Title of Project: Harnessing the potential of SIRT1 to treat acute and chronic glaucoma with gene therapy

Pharmacologic activation or genetic over-expression of the SIRT1 signaling pathway continues to show promise by preventing retinal ganglion cell (RGC) loss and axonopathy in both acute models of optic nerve damage. Evidence suggests a mechanism of action that involves upregulation of genes in responsible for increased oxygen consumption and neutralization of oxidative stress. Previous manuscripts demonstrate a role for gene therapy in improving visual and structural outcomes in a subacute model of optic neuropathy, however results were limited by transduction specificity and efficiency. We hypothesize that AAV-mediated overexpression of SIRT1 in RGCs specifically can reduce RGC loss, thereby preserving visual function. The RGC specific neuroprotective potential of RGC-selective SIRT1 gene therapy in an acute optic nerve crush (ONC) model showed promise, so we followed experimentation using a chronic magnetic microbead model of optic nerve damage. Briefly, cohorts of C57Bl/6J mice received intravitreal injections of therapeutic or control AAVs using a ganglion cell promoter, 8 weeks later, the IOP was chronically elevated was induced using a magnetic microbead model to induce optic neuropathy. Retina and optic nerves were harvested to investigate RGC survival by immunolabeling and optic nerve axon were stained and counted. AAVSNCG.eGFP and AAV-SNCG.SIRT1 vector showed a 42% and 39% efficiency, compared with a 25% (p <0.05) efficiency previously published (AAVCMV.eGFP). The magnetic microbead model demonstrated chronically elevated IOP with MB, 24±5 mmHg compared with BSS injected animals 9.8±1.2 mmHg (p<0.003) for 8 weeks. This elevated IOP corresponded to a significant reduction of visual function by optic kinetic responses (OKR; 0.160±0.098 cyc/degree) compared with BSS control injected normotensive animals 0.412±0.078 cyc/degree). This loss of visual function also manifested as a time-dependent degree in retinal ganglion cells at six weeks (BSS:1685.75±708; MB: 1019±212; p<0.05) and eight weeks (MB: 804±724; p<0.003). This loss of visual function overtime, but most prominently by 8 weeks as measured by OKR (MB, AAV-eGFP: 0.206±0.018; MB, AAV-SIRT1: 0.309±0.038; p<0.03), RGC preservation (MB, AAV-eGFP: 1026±432; MB, AAV-SIRT1: 1214±398; p<0.03), and axonal preservation (MB, AAV-eGFP: 4642±1121; MB, AAV-SIRT1: 4510±1217; p>0.03). Over-expression of SIRT1 through AAV-mediated gene transduction suggests a RGC selective component of neuroprotection which is effectively sustained using a chronic model of optic neuropathy context making it a strong and first of its kind, therapeutic candidate for use in acute, sub-acute, and chronic optic nerve diseases.

Harold Amos Medical Faculty Development Program

Name: Eugenia C. South, MD MS

Institution: Perelman School of Medicine at the University of Pennsylvania

Mentor(s): Charles Branas, PhD and Therese Richmond, PhD

College: Harvard University

Medical/Dental/Nursing PhD School: Washington University School of Medicine

Residency: Penn Medicine

Fellowship: Robert Wood Johnson Clinical Scholars Program at Penn

Title of Project: Leveraging Urban Nature to Promote Mental Health in Black Neighborhoods

**Background:** The places where people live, work, and play have a profound effect on their mental health and wellbeing. This is particularly relevant in Black neighborhoods, where historic and ongoing manifestations of structural racism serve to segregate residential environments and unequally distribute resources and risks. Black urban neighborhoods often lack quality infrastructure such as sidewalks and parks, and physical conditions such as vacant and dilapidated spaces, poor lighting, and trash are ubiquitous and unavoidable. These hazards are associated with health threats such as depression and stress. In addition, health promoting aspects the neighborhood environment – such a greenspace – is the lowest in formerly redlined, Black neighborhoods. Green space has been cited as a potential buffer between inequitable neighborhood conditions and poor health. However, there is limited evidence *how* to increase exposure to green space and *how much* exposure is needed to produce benefit. The broad objectives of this proposal are to pilot test two intervention strategies to increase green space use through the following aims:

**(SA1)** INTERVENTION DEVELOPMENT: Develop and test a person-based intervention (Nature Coach) to help people increase greenspace use.

**(SA2)** PILOT RCT: Pilot test a place-based and a person-based intervention – separately and in combination - to increase green space use and improve mental health.

**Methods:** *Aim 1.* We developed a 4-week intervention leveraging a behavioral economics framework, that included a Nature Coach, digital nudges and personalized goal feedback designed to nudge people to spend time in nearby nature. We then conducted a randomized controlled trial (RCT) among postpartum women (n=36) in Philadelphia, PA between 9/9/2019 and 3/27/2020. Nature visit frequency and duration was determined using GPS-data and mental health was measured using postpartum depression screening.

*Aim 2.* We are in the middle of conducting a factorial-designed RCT in Black neighborhoods (n=6) in Philadelphia, PA starting in 4/2021with planned completion in 8/2022. Neighborhoods were randomized to one of four arms: (a) control, (b) nature coach intervention (from aim 1), (c) community co-designed greenspace, and (d) nature coach + community greening. 73 residents have been enrolled.

**Results:** *Aim 1.* Participants were from low-income, majority Black neighborhoods. Compared to control, the intervention arm had a strong trend toward longer duration and higher frequency of nature visits (IRR 2.6, 95%CI 0.96-2.75, p=0.059). When analyzing women who completed the intervention (13 of 17 subjects), the intervention was associated with three times higher nature visits compared to control (IRR 3.1, 95%CI 1.16-3.14, p=0.025).

*Aim 2.* At baseline, participants are 64% female, 88% Black, and 66% have household incomes less than $35,000. Participants completed surveys assessing depression symptoms, resilience, and wellbeing. In partnership with a landscape architect firm, we also conducted 2-3 community meetings in each of the 3 neighborhoods receiving the community co-designed greenspace and have finalized design plans to turn previously greened vacant lots into mini park spaces.

**Next steps:** New greenspaces will be installed in Oct/Nov 2021 and we will hold a community build day in March 2022 for painting and planting. The nature coach intervention will take place in the Spring 2022. We will conduct follow up interviews with participants from April-August 2022.

**Impact of COVID-19**: We were ready to start enrolling participants in April 2020. Then COVID hit. Because of this, we postponed Aim 2 by one year.

Harold Amos Medical Faculty Development Program

Name: Mehret Birru Talabi, MD PhD

Institution: University of Pittsburgh

Mentor(s): Sonya Borrero MD MS and Megan Clowse MD MPH

College: Kenyon College

Medical/Dental/Nursing PhD School: University of Pittsburgh School of Medicine and Graduate School of Public Health

Residency: UPMC Internal Medicine

Fellowship: Rheumatology, UPMC

Title of Project: Optimizing Patient-Centered Family Planning Care for Patients with Rheumatic Diseases

**Background/Purpose:** While rheumatologists in several descriptive studies have acknowledged the importance of family planning in their care of women with rheumatic diseases, they have also identified key barriers to this care, including time constraints, competing priorities, and inadequate communication with women’s health providers. We conducted a series of focus groups composed of rheumatologists and rheumatology advanced practice providers (APPs) to synthesize their ideas for potential tools and solutions to overcome these barriers within the rheumatology clinical setting.

**Methods:** Semi-structured qualitative focus groups were conducted with rheumatologists (N=3 groups) and APPs (N=2 groups). Trained independent qualitative analysts conducted the focus groups via Zoom video conferencing. Discussions were transcribed and two trained research coordinators developed a content-based codebook. They applied the codebook to transcripts, and discrepancies adjudicated to full agreement with the principal investigator. Differences in codes between the groups by provider type were also identified. The codes were synthesized and used to conduct a thematic analysis.

**Results:** A total of 22 clinicians participated in the study, most of whom were women (75%) working within academic practice settings (60%). Clinicians had practiced rheumatology for an average of six years (range 1-17 years). Four themes emerged from the focus groups: 1) Clinicians desired patient-directed tools and resources to educate and prepare patients to discuss reproductive health issues at the rheumatology visit; 2) Most clinicians were aware of existing reproductive health resources, but desired additional training or resources around contraception and medication safety; 3) Clinicians desired tools to facilitate contact with women’s health providers to ensure early and uncomplicated access to reproductive health care (e.g., electronic consults); 4) Clinicians were less interested in using electronic health record (EHR) reminders or alerts to support family planning care, but more interested in using pre-populated text within the EHR to include in patient notes or educational information to add to patient visit summaries. Although similar ideas were generated between the APP and rheumatologist groups, the rheumatologists were generally more interested in additional training and education (e.g., continuing medical education), whereas APPs were more interested in EHR prompts and tools.

**Conclusion:** In this study, rheumatologists and rheumatology APPs from primarily academic practice settings described tools and resources that could help them to provide more consistent and higher-quality family planning care to patients with childbearing potential. Future work will focus on the development of patient-facing tools and resources to prepare patients for family planning conversations with rheumatology clinicians. Additional educational resources are needed to address providers’ knowledge gaps around contraception and medication safety in the context of pregnancy. Finally, individual health systems and practices need to prioritize the development of accessible pathways to reproductive health care for women with rheumatic disease.

Harold Amos Medical Faculty Development Program

Name: Mabel Toribio

Institution: Massachusetts General Hospital and Harvard Medical School

Mentor(s): Steven Grinspoon and Markella Zanni

College: Duke University

Medical/Dental/Nursing PhD School: Johns Hopkins University School of Medicine

Residency: University of California, San Francisco

Fellowship: Massachusetts General Hospital

Title of Project: Cardiometabolic Effects of Gender-affirming Hormone Therapy Among Transgender Women with and at Risk for HIV

**Background:** Transgender women in the United States (US) and globally face an increased risk for HIV, with HIV prevalence rates highest among African-American/Black and Latinx transgender women. While anti-retroviral therapy (ART) has significantly increased the lifespan of people with HIV (PWH) and decreased AIDS-related mortality, rates of cardiovascular disease (CVD) have increased. In addition to ART, gender-affirming hormone therapy (GAHT) is integral to the care of transgender women with HIV (WWH). However, little is known about how GAHT affects the cardiometabolic health of transgender women with and at risk for HIV.

**Methods:** We are conducting a prospective longitudinal study assessing effects of GAHT among transgender women with and at risk for HIV. Thirty transgender women will undergo abdominal and cardiac MRI/MRS, whole body DEXA, oral glucose tolerance testing, and laboratory assessments related to select coagulation parameters prior to and after 12 months of newly initiated GAHT (estrogen replacement and testosterone suppression).

**Results:** The median age of currently enrolled transgender women (N=4) is 21.5 (20.5, 22) years; and the age that these women began to identify as transgender is 15.5 (13.3, 14) years. Twenty-five percent of transgender women in this initial cohort are African-American/Black. With respect to body composition, baseline median BMI is 27.1 (23.8, 32.9) kg/m2, median total body lean mass is 63.0 (58.6, 70.2) kg and median today body fat mass is 23.9 (17.3, 30.6) kg. All transgender women in this initial cohort have normal baseline lumbar and total hip bone mineral density (data not shown) as well as insulin sensitivity [Matsuda index: 4.5 (4.1, 5.0)] and HOMA-IR: 1.7 (1.3, 2.0)]. Baseline coagulation parameters (including levels of factor II, V, VII, VII, X, Fibrinogen, Antithrombin III, von Willebrand antigen, and protein S activity) also are normal save for one participant with a low baseline factor VII activity (data not shown).

**Conclusion:** Recruitment and enrollment for this study is currently ongoing. The baseline and longitudinal results from this study will ultimately be used to investigate CVD prevention strategies tailored to transgender women with and at risk for HIV who are receiving GAHT.

Harold Amos Medical Faculty Development Program

Name: Jasmine L. Travers, PhD, RN

Institution: New York University Rory Meyers College of Nursing

Mentor(s): Bei Wu, PhD; Patricia Stone, PhD, RN, FAAN; Nicholas Castle, PhD

College: Adelphi University

Medical/Dental/Nursing PhD School: Columbia University

Fellowship: University of Pennsylvania

Fellowship: Yale University

Title of Project: Promoting Health Equity and Eliminating Health Disparities in Nursing Home Quality Measures

This project builds on previous NIH-funded pre- and postdoctoral research and aims to evaluate disparities in quality care among nursing home (NH) residents after federal regulatory changes in 2017. Health disparities are racial/ethnic differences in the quality of healthcare that are not due to clinical needs, preferences, and appropriateness of interventions. By evaluating health disparities in NH quality care measures through the use of rigorous analytical methodologies, I might be able to develop effective interventions aimed to eliminate disparities in the next steps of my program of

research. Informed by the National Quality Framework Roadmap to eliminate health disparities related to quality measurement strategies, the specific aims of this proposal are: 1) Identify racial/ethnic differences in NH quality care measures pre and post 2017 revised regulations for inspections, and 2) Identify differences in quality care measures among NHs by proportion of Black residents pre and post 2017 revised regulations. Through the use of novel methods to evaluate health disparities, findings will contribute to the evidence-base regarding methodically generated, culturally appropriate interventions that can lead to better care for older adults nationally. Additionally, by ensuring the time and support needed to solidify my program of research, this award will advance me towards becoming an independently funded nurse scientist seeking to promote health and reduce health disparities.

Harold Amos Medical Faculty Development Program

Name: E.R. Chulie Ulloa, MD, MSc

Institution: University of California Irvine

Mentor(s): Victor Nizet

College: UC San Diego

Medical/Dental/Nursing PhD School: Stanford University School of Medicine

Residency: Boston Children’s Hospital

Fellowship: Children’s Hospital of Philadelphia

Title of Project: COVID-19: Healthy School Restart

**Background:** Understanding SARS-CoV-2 infection in children is necessary to reopen schools safely.

**Methods:** We measured SARS-CoV-2 infection in 320 learners [10.5 ± 2.1(sd); 7-17 y.o.] at four diverse schools with either remote or on-site learning. Schools A and B served low-income Hispanic learners; school C served many special-needs learners; and all provided predominantly remote instruction. School D served middle- and upper-income learners, with predominantly on-site instruction. Testing occurred in the fall (2020), and 6-8 weeks later during the fall-winter surge (notable for a tenfold increase in COVID-19 cases). Immune responses and mitigation fidelity were also measured.

**Results:** We found SARS-CoV-2 infections in 17 learners only during the surge. School A (97% remote learners) had the highest infection (10/70, 14.3%, p<0.01) and IgG positivity rates (13/66, 19.7%). School D (93% on-site learners) had the lowest infection and IgG positivity rates (1/63, 1.6%). Mitigation compliance [physical distancing (mean 87.4%) and face covering (91.3%)] was remarkably high at all schools. Documented SARS-CoV-2-infected learners had neutralizing antibodies (94.7%), robust IFN-γ+ T cell responses, and reduced monocytes.

Harold Amos Medical Faculty Development Program

Name: Stanley Vance, Jr., MD

Institution: University of California, San Francisco

Mentor(s): Jae Sevelius, PhD

College: Rhodes College

Medical/Dental/Nursing PhD School: Harvard Medical School

Residency: UCSF Pediatrics

Fellowship: UCSF Adolescent and Young Adult Medicine

Title of Project: Examining Mental Health and Gender Affirmation for Black and Latinx Transgender Youth

Purpose: To quantitatively evaluate mental health symptoms and various forms of gender affirmation for Black and Latinx transgender youth (BLTY) and compare them with peers including White transgender youth and Black and Latinx cisgender youth using large school-based and clinical datasets.

Methods: For the **school-based dataset**, secondary analyses were conducted using data from the California Healthy Kids Survey with a weighted sample representative of the California’s secondary school population. The analytic sample (n=19,780) included 9th and 11th grade BLTY, White transgender youth, and Black and Latinx cisgender youth. Outcomes include past-year depressive symptoms and past-year suicidality. Psychosocial risk factors include gender-based, sexuality-based, and race-based harassment. For the **clinic-based dataset**, secondary analyses were conducted using baseline data from Trans Youth Care, a 4-site observational study monitoring medical and psychosocial outcomes for youth initiating gender-affirming hormones. The analytic sample (n=288) included BLTY and White transgender youth. Outcomes include depression, suicidality, and anxiety. Forms of gender affirmation included living full time as gender identity, history of pubertal suppression, parental acceptance, and parental non-affirmation. For analyses of each dataset, cohorts were compared using bivariate analyses and multivariable logistic regression. Moreover, for each dataset, among BLTY, associations between outcomes and psychosocial risk factors/gender affirmation were evaluated using logistic regression analyses.

Results: For the **school-based dataset,** among BLTY, the estimated prevalence of depressive symptoms and suicidality were 50% (95% CI, 44-57) and 46% (95% CI, 39-52), respectively. Logistic regression models adjusted for demographics indicated that compared to White transgender youth, BLTY had similar odds of depressive symptoms, suicidality, and all forms of harassment. With similar analyses, compared to Black and Latinx cisgender youth, BLTY youth had higher odds of depressive symptoms, suicidality, and all forms of harassment. For BLTY, all forms of harassment were associated with increased odds of depressive symptoms and suicidality. For the **clinic-based dataset, s**imilar proportions in the BLTY and White transgender youth cohorts reported depression and anxiety; BLTY had lower rates of lifetime suicidality (55%) compared to White transgender youth (73%; p=0.003). Both cohorts had had similar Perceived Parental Acceptance scores; BLTY had higher Perceived Parental Non-Affirmation scores. Similar proportions were living fulltime as their affirmed gender and had a history of pubertal suppression. For BLTY, living fulltime as their affirmed gender decreased odds for depression; parental non-affirmation increased odds of depression and anxiety; and no history of pubertal suppression increased odds for depression, suicidality, and anxiety.

Conclusions: BLTY have high rates of mental health symptoms and are relatively vulnerable when compared with their peers. Harassment and various forms of gender affirmation should be targeted to support their mental health. Qualitative interviews with BLTY and their parents could elucidate culture-based factors and intersectional stigma that impact the mental health and gender affirmation of this population.

Harold Amos Medical Faculty Development Program

Name: Joshua Vásquez, MD

Institution: University of California - San Francisco

Mentor(s): Joel Ernst, MD, Peter Hunt, MD

College: University of Wisconsin - Madison

Medical/Dental/Nursing PhD School: University of California - San Francisco

Residency: University of Colorado - Denver

Fellowship: University of California - San Francisco

Title of Project: Role of lung myeloid cells in TB and HIV

Lung myeloid cells are widely accepted as major contributors to the pulmonary immune response to *Mycobacterium tuberculosis* (Mtb). Within the Mtb infected lung the population of myeloid cells is comprised of phenotypically distinct subsets with a differential capacity to kill, restrict, or permit growth of Mtb. Co-occurring conditions such as HIV that dysregulate myeloid cells may impair their capacity to control Mtb and contribute to poor outcomes. Since pathology from pulmonary tuberculosis (TB) occurs within the lung parenchyma, investigation of these host-pathogen interactions has been limited. Moreover, evaluation of these relationships *in situ* may be particularly valuable since the complex architecture of the granuloma is thought to provide sanctuary for reservoirs of replicating bacilli during treatment, though the mechanisms remain unclear. While impaired drug penetration in the granuloma has been described, immune and physiochemical pressures within distinct lesional micro-environments likely contribute by influencing the physiologic state of local bacteria, thereby impacting the response to treatment. To understand these relationships, we have developed a platform using a novel pharmacodynamic marker of bacterial growth based on a fundamental aspect of Mtb physiology, ribosomal (rRNA) synthesis. Visualization of Mtb rRNA synthesis *in situ* enables quantitative mapping of physiologically distinct bacterial populations in tissue lesions. Using this approach, we provide, for the first time, a spatially integrated evaluation of bacterial growth states in the granuloma and define the impact of individual drugs on their distribution. Specifically, we have identified populations of replicating bacteria disproportionately found within layers of myeloid cells of the granuloma before and during treatment. Mechanistically defining the host-pathogen relationships that support bacterial growth in the face of treatment may uncover new targets for host-directed therapies. Overall, these results support our platform as a desperately needed pharmacodynamic tool for pre-clinical drug development and investigation of host-pathogen relationships within difficult to access tissue micro-environments.

Harold Amos Medical Faculty Development Program

Name: Juan Vasquez

Institution: Yale School of Medicine

Mentor(s): Ranjit Bindra, MD, PhD

College: Harvard University

Medical/Dental/Nursing PhD School: The Warren Alpert Medical School of Brown University

Residency: The Warren Alpert Medical School of Brown University

Fellowship: Yale School of Medicine

Title of Project: Exploiting oncometabolite-induced DNA repair defects in the treatment of cancer

**Background:** Loss-of-function mutations in genes encoding the Krebs cycle enzymes fumarate hydratase (*FH*) and succinate dehydrogenase (*SDH*) induce excess accumulation of fumarate and succinate, respectively. Germline mutations in *FH* predispose patients to Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC)-associated RCC. Similarly, loss-of-function alterations of *SDH*, most commonly *SDHB*, are associated with *SDH*-deficient neoplasms, including RCC. FH and SDHBdeficient RCC tends to be aggressive and metastasize early with very limited treatment options. We have previously demonstrated that fumarate and succinate competitively inhibit αKG-dependent dioxygenases, including Lysine-specific demethylase 4A/B (KDM4A/B), leading to suppression of the homologous recombination DNA repair pathway and enhanced sensitivity to poly ADP-ribose polymerase (PARP) inhibition. We have previously demonstrated that elevated levels of fumarate and succinate both suppress the homologous recombination (HR) DNA-repair pathway. In this study, we sought to identify novel treatment approaches that exploit genomic instability in *FH*- and *SDHB*-deficient RCC.

**Methods:** CRISPR/Cas9 was used to engineer isogenic *Fh1*- and *Sdhb*-deficient murine models of RCC. The efficacy of PARP inhibition and temozolomide (TMZ), alone and in combination, was evaluated both *in vitro* and *in vivo*.

**Results:** Here, we have developed new syngeneic *Fh1*- and S*dhb*-deficient murine models of RCC. We demonstrate that *Fh1*- and *Sdhb*-deficient cells accumulate fumarate and succinate leading to an increase in unresolved DNA double-strand breaks (DSBs). Combination treatment with PARP inhibition and TMZ results in marked *in vitro* cytotoxicity in *Fh1-* and *Sdhb*-deficient cells. *In vivo*, treatment with standard dosing of the PARP inhibitor BGB-290 and low-dose TMZ significantly inhibits tumor growth without a significant increase in toxicity.

**Conclusion:** Taken together, these findings provide the basis for a novel therapeutic strategy exploiting HR deficiency in *Fh1* and *Sdhb*-deficient RCC with combined PARP inhibition and low-dose alkylating chemotherapy. Furthermore, the development of Kreb-cycle-deficient syngeneic mouse models provides a tool for future pre-clinical immunotherapy studies.

Harold Amos Medical Faculty Development Program

Name: Jason Watts

Institution: National Institute of Environmental Health Sciences

Mentor(s): Vivian Cheung

College: University of Pennsylvania

Medical/Dental/Nursing PhD School: University of Pennsylvania

Residency: Internal Medicine, Duke University

Fellowship: Nephrology, University of Michigan

Title of Project: RNA Polymerase Pausing Regulates Renal Gene Expression

**Background:**  This project studies how RNA polymerase pausing is regulated and its effect on gene expression in the kidney. Synthesis of RNA is a discontinuous process where RNA polymerase undergoes punctuated pauses during RNA chain elongation. We found that RNA Polymerase II (RNAPII) pauses in a highly regulated manner. At over 1,000 genes, RNAPII paused at the same nucleotide locations across individuals and human cell types. This finding enabled us to identify elements in the nucleic acid sequence that contribute to the precise regulation of RNA polymerase pausing. Here, using mitochondrial transcription as a model system, we examine the role of nucleic acid structure in the regulation of RNA polymerase pausing, gene expression, and cellular function.

**Results:** The mitochondrial genome encodes proteins that are required for energy production in eukaryotic cells and these essential genes are transcribed by the mitochondrial RNA polymerase (mtRNAP). To characterize mtRNAP pausing, we used a transcription run-on technique (PRO-seq), which maps active RNA polymerase at high resolution. In primary fibroblast cells from different individuals, we found that as the polymerase transcribes the mitochondrial genome it pauses over 400 times at consistent locations. These brief stops occur most often after mtRNAP has transcribed through guanine (G)-rich regions. Using computational and experimental approaches, we found that the G-rich sequences can form guanine (G)-quadruplexes, which are secondary structures that are stabilized by non-canonical base pairing between guanine residues. We treated fibroblasts with a drug (RHPS4) that stabilizes G-quadruplexes and we found that transcription by mtRNAP was reduced due to more frequent pausing. This resulted in significantly lower expression of mitochondrial-encoded genes and consequently decreased ATP generation. Renal proximal tubule cells primarily act to reclaim solutes from the glomerular filtrate, and they require ATP from mitochondria for transporter function. We treated proximal tubule cells with RHPS4 to stabilize G-quadruplexes and found significantly reduced transporter function due to loss of mitochondrial ATP production.

**Conclusion:** We find that mtRNAP pausing is mediated by guanine-rich sequences which form guanine-quadruplex secondary structures. G-quadruplex mediated mtRNAP pausing regulates mitochondrial gene expression, mitochondrial ATP production, and the function of renal proximal tubule cells. In future studies, we will examine how proximal tubule cells regulate the abundance of mitochondrial G-quadruplexes to tune transcriptional output to meet the energy demands in the cells.