Harold Amos Medical Faculty Development Program

Name: Ade Adamson, MD, MPP

Current Institution: Dell Medical School at the University of Texas at Austin

Mentor(s): Dr. Michael P. Pignone, Dr. H. Gilbert Welch

College: Morehouse College

Medical/Dental/Nursing PhD School: Harvard Medical School

Residency: University of Texas Southwestern

Fellowship: N/A

Title of Project: Elucidating the scope and potential harms of melanoma overdiagnosis

**Project Background:** The incidence of cutaneous melanoma has been rising rapidly in the US; however, a commensurate increase in mortality due to melanoma has not been observed. These trends suggest overdiagnosis is occurring, less is known about the scope and associated harms.

Aim 1: Estimate the proportion of melanoma overdiagnosed in the US (**JAMA Derm 2022**)

**Design, Setting, and Participants:** This cohort study used joinpoint regression of Surveillance, Epidemiology, and End Results data from 1975 to 2014 to determine melanoma incidence and mortality trends among Black and White patients in the US. Using trends in mortality due to melanoma in Black patients as a marker for improvements in medical care, the expected mortality trends in White patients if medical care had not improved were estimated. This served as a marker for the change in true cancer occurrence. Overdiagnosis was calculated as the difference between observed incidence and estimated true cancer occurrence.

**Primary Outcome:** Proportion of melanoma overdiagnosed among White patients in 2014.

**Results:**  From 1975 to 2014, melanoma incidence increased approximately 4-fold in White women (incidence rate ratio [IRR], 4.01 [95% CI, 3.65-4.41]) and 6-fold in White men (IRR, 5.97 [95% CI, 5.47-6.52]), whereas it increased less than 25% in Black women (IRR, 1.21 [95% CI, 0.97-1.49]) and men (IRR, 1.17; [95% CI, 0.77-1.78]). Mortality due to melanoma decreased approximately 25% in Black women (morality rate ratio [MRR], 0.76 [95% CI, 0.63-0.90]) and men (MRR, 0.72 [95% CI, 0.62-0.84]), was stable in White women (MRR, 1.02 [95% CI, 0.96-1.09]), and increased almost 50% in White men (MRR, 1.49 [95% CI, 1.25-1.77]). Based on these trends, an estimated 59% (95% CI, 45%-70%) of White women and 60% (95% CI, 32%-75%) of White men with melanoma were overdiagnosed in 2014.

**Conclusions:** The discrepancies in incidence and mortality trends found in this cohort study suggest considerable melanoma overdiagnosis among White patients in the US.

Aim 2: Estimate cost of in situ and early invasive melanoma in the US **(Collecting data)**

Nearly half of melanomas diagnosed in the US are in situ and many of these may be overdiagnosed. Melanoma diagnosis produces treatment costs, increased follow up for disease surveillance, and the risk for repeat diagnosis. Using claims data, we will estimate the health care utilization costs associated with in situ and early invasive melanoma.

Aim 3: Explore the lived experience of patients with invasive melanoma and melanoma in situ (MIS) to determine how these diagnoses affect quality of life. (**Preliminary results**)

**Methods:** Through surveys and semi-structured interviews with patients with MIS or early invasive melanoma we will explore if there are key differences in quality of life (QOL) related to diagnosis, treatment, and survivorship.

**Results:** Many patients expressed feelings of guilt, self-blame, negative changes in body image, dread before follow-up appointments, and fear or cancer recurrence.

**Conclusions:** Receipt of a diagnosis of MIS or early invasive melanoma can have a profound impact on patient quality of life despite highly favorable prognosis of >99% 5-year survival.

Harold Amos Medical Faculty Development Program

Name: Daniel Addison, MD

Institution: Ohio State University

Mentor(s): William Abraham MD, John Byrd MD, Orlando Simonetti PhD

College: Oral Roberts University

Medical School: Meharry Medical College

Residency: Baylor College of Medicine

Fellowships: Baylor College of Medicine (Cardiology); Massachusetts General Hospital (Advanced Cardiovascular Imaging, and Cardio-Oncology)

Title: Defining and Predicting Tyrosine Kinase Inhibitor Cardiotoxicity in Hematologic Cancer Patients

**Background:** Nearly 300,000 Americans are affected by chronic lymphoma leukemia (CLL). Ibrutinib, a novel oral Bruton’s tyrosine kinase inhibitor (BTKI), dramatically improves outcomes in CLL and various hematologic malignancies and is now approved for lifelong therapy, with >300 additional trials testing ibrutinib and/or other next generation BTKIs ongoing. However, BTKIs associate with a >5-fold risk of cardiac arrhythmias, including atrial fibrillation (AF) and potentially life-threatening ventricular arrhythmic (VA) events. In preclinical models, these events are paralleled or proceeded by diffuse myocardial injury (inflammation and fibrosis). Yet, whether this is seen in patients, or has implications for future cardiotoxic risk is unknown.

**Methods:** Leveraging large cohort(s) of consecutive hematologic malignancy patients receiving BTKIs, we retrospectively evaluated the cardiac phenotype of ibrutinib-cardiotoxicity, using cardiovascular magnetic resonance imaging (CMR). We also evaluated the effect of next-generation BTKI-therapy, acalabrutinib, on arrhythmia risk. Observed measures are compared with similar aged cardiovascular disease (CVD)-risk matched cancer controls; and to general population rates. Finally, in an ongoing observational trial, we prospectively define the time-dependent relationship and structural effect of BTKI use on myocardial injury and other cardiac remodeling (ie. atrial and vascular). Multivariable regression was employed to assess evaluate the factors associated with injury/remodeling and arrhythmic risk.

**Results:** Among patients with suspected cardiotoxicity referred for CMR (n=33), nearly two-thirds had evidence of myocardial injury, including >50% with ventricular scar. This patterned remained after accounting for traditional cardiac risk factors; and those with injury saw up to a 5-fold increase in future cardiotoxic events (HR: 4.9, *P*=0.04; arrhythmias, heart failure, and sudden death); (*JAMA: Onc*, 2022). Concurrently, among 290 patients, we found the use of acalabrutinib associated with a ~3-fold increase in incident AF (*P*<0.001), and >8-fold increase in VA and sudden death events (*P*<0.001), accounting for traditional CVD risk (*Blood*, 2022). We also observed an increase in hypertensive risk (*JHO*, 2022). These key themes inform our ongoing pilot CMR trial (approaching one-third of target enrollment; target n=50), whereby we now include patients receiving next generation BTKIs, to more thoroughly define the structural mechanisms behind cardiotoxic risk in patients treated with this class of drug.

**Conclusions (interim):** CLL patients receiving BTKI therapy face a high risk for serious cardiotoxic events. Among those with ibrutinib-associated cardiotoxicity, myocardial injury is common. Continued study is needed to define the early risk factors, and mechanisms involved in these events.

**Impact of COVID-19:** We were delayed in IRB approval and initial enrollment. Because of this, the trial was delayed by roughly 12-15 months. I continued to publish from the registry/ retrospective cohort (ex. Blood, 2022). Enrollment of participants is now ongoing.

Harold Amos Medical Faculty Development Program

Name: Diana Alba, MD

Current Institution: University of California - San Francisco

Mentor(s): Suneil Koliwad MD, PhD, Peter Hunt MD.

College: Georgia Institute of Technology

Medical/Dental/Nursing PhD School: New York Medical College

Residency: University of Miami/Jackson Memorial Hospital

Fellowship: University of California, San Francisco

Title of Project: Understanding the Role of Adipose Tissue Fibrosis in Metabolic Disorders, Inflammation, and in HIV Infection.

**Background:**

People living with HIV (PLWH) can survive decades on antiretroviral therapy, but this success is often accompanied by a disproportionate burden of metabolic disease, including type 2 diabetes (T2D). In PLWH, the risk of T2D is nearly 3-fold higher than the general population in the U.S., an increase not readily explained by traditional risk factors including obesity. While a few inflammatory factors have been associated with insulin resistance and T2D in treated HIV, the immunologic pathways that may drive these complications remain unclear. We aimed to investigate the interplay between adipose tissue fibrosis, alterations in various inflammatory pathways, metabolism, and insulin resistance, in the context of HIV infection.

**Methods**:

We use case-cohort design where we randomly sampled all Centers for AIDS Research (CFAR) Network of Integrated Clinical Systems (*CNICS)* cohort participants with available plasma after 1 year of ART-mediated viral suppression, and from the same timepoint, all participants who were subsequently diagnosed with an T2D (all centrally adjudicated). The relationship between 13 plasma biomarkers normalized to the cohort interquartile range (IQR) and subsequent event risk was assessed by logistic regression, adjusting for age, natal sex, nadir CD4, and other potential confounders (smoking, IDU, and HCV history). We then studied possible mechanisms by analyzing glucose tolerance test data, body composition data, and adipose tissue samples from the UCSF SCOPE cohort. We assessed the relationship between the same plasma markers measured in the CNICS cohort, insulin resistance (HOMA-IR) and the fibrosis marker hydroxyproline in subcutaneous adipose tissue (SAT) aspirates in ART-suppressed PLWH and those without HIV, all without T2D and frequency matched by HgA1 in the SCOPE cohort.

**Results**: Among 843 (9,340 eligible) ART-suppressed CNICS participants, there were 97 incident T2D cases. Median age was 46, 84% were men, and 16% had a history of HCV. median current and nadir CD4 were 571 and 250. Higher IL-6, IL-18, IP-10, sCD163, suPAR, sTNFR2 and kynurenine-to-tryptophan (KT ratio) were associated with incident T2D(Figure). The adipose tissue sampling study included 41 PLWH and 31 sero-negative participants, 68% men with median values: age, 50 years; BMI, 28 kg/m2; HgA1c 5.4%. Compared to those without HIV, PLWH had higher SAT levels of the fibrosis marker hydroxyproline (P=0.03) and higher plasma KT ratio (P=0.03). While most inflammatory markers predicted HOMA-IR in PWH, KT ratio was the most strongly correlated with adipose tissue hydroxyproline levels (rho: 0.31, P=0.04). Adjustment for KT ratio also significantly attenuated the difference in hydroxyproline levels between people with and without HIV.

**Conclusion**: Many inflammatory pathways, including the kynurenine pathway of tryptophan catabolism, predict incident T2D in treated HIV infection. PLWH also have abnormally high SAT fibrosis, which is also associated with the kynurenine pathway. As the kynurenine pathway has been linked to Treg expansion and fibrotic pathways in prior studies, these data may suggest a potential role of the kynurenine pathway in subcutaneous adipose tissue fibrosis and insulin resistance in treated HIV.

Harold Amos Medical Faculty Development Program

Name: Edilberto Amorim, MD

Current Institution: UCSF

Mentor(s): Sri Nagarajan, PhD

College: N/A

Medical/Dental/Nursing PhD School: Escola Bahiana de Medicina e Saúde Pública

Residency: Neurology, University of Pittsburgh

Fellowship: Neurocritical Care and Epilepsy, Massachusetts General Hospital and Brigham and Women’s Hospital

Title of Project: Brain Edema in Hypoxic-Ischemic Brain Injury

**Introduction:** Severe brain injury impacts neurological recovery for more than 100,000 Americans surviving cardiac arrest every year.Brain edema is a complication from hypoxic-ischemic brain injury in near half of these patients, but no brain physiology target is available for treatment titration in high-risk patients.

**Methods:** Retrospective analysis involving 11 academic centers in the U.S. Patients with cardiac arrest who were unable to follow commands on admission and underwent continuous EEG and brain MRI for prognostication were included. Unfavorable EEG was defined as EEG showing suppressed, burst suppressed, or generalized periodic discharges. Poor outcome at time of hospital discharge was defined as a Cerebral Performance Category of 3-5 (i.e., dependent for activities of daily living to dead). Diffusion weighted MR images (DWIs) were quantitatively evaluated using a custom image processing pipeline implemented in Python 3.7. XGBOOST, Random Forests, and SHAP algorithms were used for outcome prediction. We pursued a leave-one-hospital out analysis to evaluate variability in model performance.

**Results:** We have identified 664 comatose subjects with cardiac arrest who underwent both continuous EEG monitoring and brain MRI. Guideline-based prognostication identified poor outcome in 125 (19%) subjects with 100% specificity, but 509 (77%) had indeterminate prognosis. The AUC for poor outcome prediction was 0.84 with unfavorable EEG and brain injury on MRI being the most relevant features. We completed quantitative regional analysis of diffusion weighted imaging for 274 subjects, which demonstrated a strong association between unfavorable EEG and brain edema severity (p<0.001). Prediction performance of poor outcome using clinical information and quantitative MRI data had large variability depending on algorithm and center tested (AUC range from 0.61 to 0.91).

**Conclusions:** Most patients with cardiac arrest have indeterminate prognosis despite use of multimodal tools. Ensemble tree models combining qualitative and quantitative data have good performance, however there is high variability in performance likely due to population and clinical practice heterogeneity.

Harold Amos Medical Faculty Development Program

**Name**: Juan Pablo Arroyo Ornelas

**Current** **Institution**: Vanderbilt University Medical Center

**Mentor(s)**: Raymond C. Harris and Gautam Bhave

**College**: Universidad La Salle, Mexico City, Mexico

**Medical/Dental/Nursing PhD School**: Universidad La Salle and Universidad Nacional Autonoma de Mexico (UNAM), Mexico City, Mexico

**Residency**: Internal Medicine - Vanderbilt University Medical Center

**Fellowship**: Nephrology – Vanderbilt University Medical Center

**Title of Project**: Kidney-derived vasopressin and its role in polycystic kidney disease

**Background:** Vasopressin has traditionally been thought to be produced by the neurohypophyseal system and then released into the circulation where it regulates water homeostasis. The syndrome of inappropriate secretion of anti-diuretic hormone (vasopressin) occurs is present in multiple clinical scenarios including cancer, pulmonary disease, trauma, and infections. Moreover, in polycystic kidney disease (PKD) vasopressin drives cyst growth and progression of disease. These observations raised the question if vasopressin could be produced outside of the brain, whether the kidney could be a source of vasopressin and if kidney-derived vasopressin could play a role in PKD.

**Results:** To study the local production of vasopressin by the kidney we searched publicly available RNAseq data sets and found that numerous studies reported expression of vasopressin in kidney epithelial cells. We then confirmed expression of vasopressin mRNA in collecting duct cells in vitro and found that vasopressin mRNA expression increases with salt induced hypertonic stress. We also used qPCR and RNA in situ hybridization and confirmed the expression of vasopressin mRNA in mouse and human kidneys. We then utilized a reporter cell line with cyclic AMP sensitive luciferase to confirm the presence of biologically active vasopressin in the collecting duct cell medium. The vasopressin protein is a highly processed peptide that is secreted as a nine amino-acid peptide. Therefore, to confirm local production of vasopressin protein we generated an antibody that targets the pre-pro-vasopressin protein. We found that collecting duct cells in vitro make vasopressin and that vasopressin is found both in the cytoplasm and in Rab3+ secretory vesicles. We confirmed that pre-pro-vasopressin protein is found in both mouse and human collecting duct cells and it increases in mice after water restriction. We then obtained samples of non-diseased human kidneys and kidneys with PKD and found that pre-pro-vasopressin protein is significantly upregulated in human kidneys with PKD.

**Conclusion:** Together our data shows that kidney tubular epithelial cells produce biologically active vasopressin and that kidney-derived pre-pro-vasopressin protein expression in increased in kidneys with PKD.

Harold Amos Medical Faculty Development Program

Name: Lorel E. Burns, DDS, MS

Current Institution: New York University College of Dentistry

Mentor(s): Heather T. Gold, PhD

College: University of Pennsylvania

Medical/Dental/Nursing PhD School: New York University College of Dentistry

Residency: University of Pennsylvania School of Dental Medicine

Fellowship: N/A

Title of Project: *Endodontic Treatment of Children and Adolescents: Access to Care and Procedural Outcomes*

**Background:** Children and adolescents experiencing dental pain and infection that require endodontic treatment in permanent teeth may present with unique root canal anatomyand behavioral considerations, compared to adults.These differences often require distinct considerations and expertise, such as the ability to manage the behavior of children and adolescentsand the required proficiency to perform the technical aspects of endodontic treatment. There are apparent gaps in ability of pediatric patients to access these treatments.

The objective is to fill in gaps in knowledge concerning accessibility and outcomes of endodontic treatment of children and adolescents in the United States. Dr. Burns’ concurrent NIH/ NIDCR grant examines the provision and procedural outcomes of conventional endodontic treatment, non-surgical root canal therapy, with a mixed-method approach. To complement that grant and fulfill the objective, the AMFDP award supports specific studies of the endodontic procedures intended for necrotic immature permanent teeth (apexification; revascularization/ regeneration) as well as vital pulp therapy procedures (direct and indirect pulp capping; apexogenesis).

**Methods:** Administrative claims data from New York State (public-payer) and Massachusetts (all payer) were used to identify the provision of the endodontic procedures as well as perform a retrospective, longitudinal cohort study to assess procedural survival and the occurrence of subsequent adverse events. Additionally, a national survey will be distributed to over 6,000 endodontists and pediatric dentists to ascertain their treatment planning considerations and referral patterns for endodontic treatment of immature permanent teeth.

**Preliminary Findings**: The provision of the revascularization/ regeneration, indirect pulp cap, and apexogenesis procedures were so infrequent in both the New York State and Massachusetts data sets that the analysis of procedural outcomes could only be performed on the apexification and direct pulp cap procedures. Gaps in insurance coverage for these procedures is suspected to be the reason they appear infrequently in the data sets. Apexification procedures (n=116) performed on public-payer beneficiaries from New York State had a 1-year survival rate of 94.0% [95% CI: 89.7-98.4] and a 3-year survival rate of 85.7% [95% CI: 79.4-92.6]. Direct pulp cap procedures (n= 1,518) performed in New York and Massachusetts had a 1-year survival rate of 92.3% [95% CI: 91.0-93.6] and a 3-year survival rate of 85.4% [95% CI: 83.5-87.4]. In Massachusetts, the direct pulp cap procedure is not covered by Medicaid and the data set was composed entirely of enrollees of private-payer insurance. Statistically significant differences in treatment outcomes of vital pulp therapy were observed by state/ payer type (p=0.041).

**Next Steps:** The national survey will be distributed in the first quarter of 2023.

Harold Amos Medical Faculty Development Program

Name: Tamryn F. Gray, PhD, RN, MPH

Current Institution: Dana-Farber Cancer Institute and Harvard Medical School

Mentor(s): James Tulsky, MD and Charlotta Lindvall, MD, PhD

College: UNC-Chapel Hill, Harvard T.H. Chan School of Public Health

Medical/Dental/Nursing PhD School: Johns Hopkins University

Residency: N/A

Fellowship: Dept of Psychosocial Oncology and Palliative Care, Dana-Farber Cancer Institute

Title of Project: Family Forward: Evaluating the Implementation and Impact of the CARE Act – Progress of a Mixed Methods Study

**Project Summary:** While family caregivers are critical during care transitions across health settings, many feel ill prepared for their role at the time of hospital discharge—a pivotal time fraught with potential gaps in care and services that can put patients at risk for unplanned readmissions. Despite the important role of caregivers at discharge, there is currently no standard for integrating them into the plan of care. The Caregiver, Advise, Record, and Enable (CARE) Act and proposed Medicare regulations require caregiver integration into the discharge planning process. Though it may vary by state, the CARE Act generally requires that hospitals: (1) advise patients of their opportunity to identify a caregiver, (2) record the caregiver’s name and contact information in the health record, and (3) enable caregivers by providing as much notice as possible about discharge timing and instructions. Implementing the CARE Act may require additional time and resources on caregiver education and training, but could improve patient outcomes, reduce unplanned readmissions, and lower costs. To date, data are lacking about the CARE Act implementation. The goal of the study is to evaluate the implementation and impact of the CARE Act to better prepare caregivers of older adults in hospital discharge planning.

**Progress Report:**

***Specific Aim 1:*** To determine EHR documentation of caregiver involvement and factors associated with documentation in seriously ill older adults using NLP and structured data fields. To date, we have established cohort determination based on the criteria that patients are >65 years old and admitted into one of the five hospitals within the Mass General Brigham health system. We used ICD-10 codes to define admission for surgical procedures, including severe trauma, colectomy, hip replacement, knee replacement, coronary artery bypass, and lower extremity revascularization. These individuals were included if they had at least one serious illness (based on Dartmouth Atlas criteria and ICD-10 codes) and continuous fee-for-service Medicare enrollment for 12 months before and after surgical discharge or until death. Next steps include using a validated annotation method to generate a labeled dataset related to caregiver involvement for machine learning NLP. We will split 80% dataset as the training set and remaining 20% as the validation set. The best model parameter will be based on the validation set ROC-AUC score.

***Specific Aim 2:*** To assess the impact of EHR documentation of caregiver involvement on healthcare utilization (e.g., palliative care services and unplanned readmission rates) and costs in seriously ill older adults using Medicare claims data combined with EHR data. Next steps include linking the cohort with Medicare data, prepare cohort information including covariates, and link Medicare data for NLP and machine learning. We will use descriptive statistics to examine factors associated with caregiver documentation and regression tests to examine associations between caregiver documentation and 30-day readmission rates and costs controlling for clinical (diagnosis, length of hospital stay) and demographic factors (patient age, sex, race, insurance status, comorbidities, zip code, income).

***Specific Aim 3:*** To understand the strategies, facilitators, barriers, and opportunities in CARE Act implementation from the perspectives of hospital staff and community partners. I have developed the content for the interview guide, informed by the Consolidated Framework for Implementation Research (CFIR), and currently receiving feedback from mentors prior to submitting an IRB amendment. Upon approval, we will begin participant recruitment shortly thereafter.

**Harold Amos Medical Faculty Development Program**

**Name:** Lisa McElroy MD MS

**Institution:** Duke University

**Mentor(s):** L Ebony Boulware MD MPH

**College:** The Evergreen State College

**Medical/Dental/Nursing PhD School:** Michigan State University College of Human Medicine

**Residency:** Medical College of Wisconsin

**Fellowship:** University of Michigan

**Title of Project:** Living Donor Kidney Transplant: Identifying the Phenotype of an Equitable Program

**Abstract:**

Project Summary: Of the treatment options for end stage kidney disease (ESKD), living donor kidney transplant (LDKT) has several advantages including decreased waiting time, improved patient reported quality of life, and superior long term graft survival. LDKT is significantly underutilized by black patients, despite a prevalence of ESKD nearly 4 times that of Caucasians. Knowledge of this racial disparity is longstanding, yet the rate of LDKT in AAs has declined over the last decade and the racial disparity in LDKT has worsened. Although patient-level drivers of racial disparities in LDKT have been identified, the influence of transplant center policies and processes of care on racial disparities in LDKT is unknown. My central hypothesis is that care processes at transplant centers with lowest racial disparity in LDKT rates can be systematically assessed, consolidated and disseminated to other programs to advance the field toward eliminating persistent racial disparities in LDKT. The objective of this project is to identify practices at US transplant centers that can be disseminated to reduce bias and promote equity in living donor kidney transplant through quantifying racial disparities in LDKT rates among US transplant centers (Aim 1); characterizing the organizational structure, program components, and care processes among transplant centers with highest and lowest racial disparity in LDKT rates through an assessment of LDKT program websites, a web-based questionnaire of LDKT program leadership and semi-structured interviews with transplant center administrative leaders (Aim 2); and identifying ‘best practices’ among US transplant centers with the lowest racial disparity in LDKT rates (Aim 3).

Progress Report: I applied an implementation science framework, the Pragmatic Robust Implementation and Sustainability Model (PRISM), to racial equity in living donor kidney transplantation. The applied PRISM framework provides a foundation for identification of multi-level determinants of racial inequities across the spectrum of LDKT care, and I used this framework to organize my study data collection. I performed a quantitative analysis of the Health Resources Services Administration, United States Renal Data System and the Scientific Registry of Transplant Recipients. I found on multivariable analysis that annual LDKT black:white rate ratios ranged from 0.2197 to 0.2894, indicating that racial inequity in center-level LDKT Improved over 11 years, but was not achieved. Factors most negatively associated with LDKT rates for Black patients included interquartile range of the area deprivation index and lack of state Medicaid expansion. These results allowed us to identify the highest and lowest performing LDKT centers in the US for further study. I additionally examined the quality of living donor kidney transplant (LDKT) websites with respect to principles of health equity. Among the 185 transplant center websites reviewed, only 14.6% of LDKT sites could be accessed directly from the main transplant center webpage. The overall Readable score was 3.0±0.7 and the mean Flesch-Kincaid grade level and ease score were 9.1±1.3 and 50.7±7.9, respectively. The median SAM was 45.6±5.5. Adherence to equity-focused principles of web design was correlated with LDKT black-to-white patient ratio (r=0.25), total kidney transplant volume (r=0.21), DDKT volume (r=0.2), LDKT volume (r=0.19), and LDKT-to-total transplant ratio (r=0.12). I also distributed a web-based questionnaire to LDKT medical and surgical program directors to characterize unique screening and evaluation processes at transplant centers that may facilitate LDKT in racial and ethnic minorities. I am continuing to collect results as I construct the guide for semi-structured interviews with transplant center administrative leadership, which I will complete this fall and winter. I am also beginning to plan site visits to transplant centers with the highest racial equity in LDKT next year.

Harold Amos Medical Faculty Development Program

Name: Jose Ricardo “Ricky” McFaline-Figueroa, MD, PhD

Current Institution: Dana-Farber Cancer Institute

Mentor(s): Jean Zhao, PhD; Patrick Wen, MD

College: University of Puerto Rico at Mayaguez

Medical/Dental/Nursing PhD School: Columbia University

Residency: Mass General Brigham Neurology Residency Program

Fellowship: DFCI-MGH-BWH Neuro-Oncology Fellowship Program

Title of Project: *Exploring the role of the polycomb repressor complex 2 in glioblastoma immune evasion*

Glioblastoma (GBM) is the most common malignant primary brain tumor in adults and unfortunately, a universally fatal form of brain cancer (Ostrom et al. 2018). Clinical trials of immune checkpoint inhibitors (ICIs) as monotherapy in GBM have resulted in low response rates. However, those who derived a benefit from immunotherapy had significantly more durable responses compared to standard-of-care (Reardon et al. 2017). Thus, strategies that sensitize GBM to immunotherapy are in high demand. The epigenetic regulator polycomb repressor complex 2 (PRC2) is associated with downregulation of antigen processing and presentation machinery (APM) in pediatric high-grade gliomas and is a mechanism of tumor-intrinsic immune evasion in several cancer types (Krug et al. 2019, Burr et al. 2019). We sought to determine whether the PRC2 plays a role in adult GBM immune evasion and whether inhibition of PRC2 could affect the susceptibility of GBM to immune checkpoint blockade. Using a published dataset (Neftel et al. 2019), we find that expression of the catalytic subunit of PRC2, EZH2, and DNMT1 are inversely correlated to APM expression in GBM tumor tissues analyzed by single-cell RNA seq. We also find that pharmacologic inhibition of PRC2 or DNMT1 in GBM patient-derived cell lines (PDCLs) *in vitro* results in a large increase in the expression of APM genes by RT-PCR. This transcriptional change correlates with a decrease in the number of GBM PDCLs expressing little-to-no class 1 human leukocyte antigen (HLA) complex on their cell surface after *in vitro* treatment. To determine whether these expression changes increase the susceptibility of GBM to immune checkpoint blockade, we are studying the effect of inhibition of PRC2 with the EZH2 inhibitor tazemetostat alone and in combination with anti-PD1 antibody on two genetically-engineered mouse models of GBM which similarly upregulate APM upon PRC2 inhibition *in vitro* (Pten-/-TP53-/- mice and CDKN2A/B-/-Pten-/-EGFRvIII mice) and a humanized mouse model of GBM (huNBSGW-BT112). Finally, we are analyzing human glioblastoma tumor tissue to determine the spatial relationship between single tumor cells expressing high or low protein levels of EZH2 or its catalytic product H3K27me3, and their proximity to immune cell subtypes using a highly multiplex immunofluorescence technique, tissue-based cyclic immunofluorescence (t-CyCIF). We hope that this work will uncover strategies to increase the efficacy of immune checkpoint blockade and result in long-term responses in patients with GBM.

Harold Amos Medical Faculty Development Program

Name: Emilio Ramos

Current Institution: UCSF

Mentor(s): Arun Wiita

College: UCSD

Medical/Dental/Nursing PhD School: UCLA/Charles Drew Program

Residency: UCSF Anatomic Pathology

Fellowship: UCSF Hematopathology

Title of Project:Profiling the Cell Surface of MPN Blast Phase Suggests Unique Biology and Therapeutic Targets

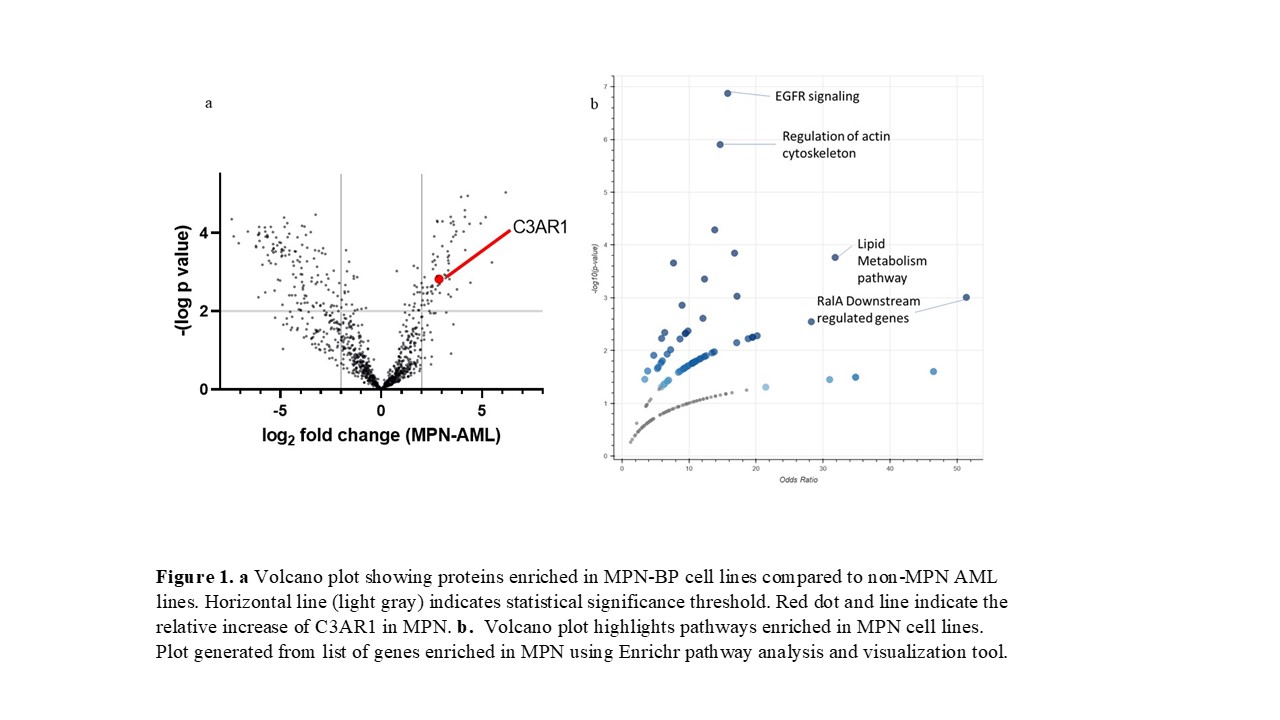
**Background:** Myeloproliferative neoplasm in blast phase (MPN-BP) is a secondary acute myeloid leukemia (sAML) notable for poor response to chemotherapy and short median survival. In a prior study, we used glycoprotein capture cell surface proteomics to find CD72 as a novel immunotherapy target highly expressed in *KMT2A*-rearranged B lymphoblastic leukemia, the poorest-prognosis subtype of this disease (Nix et al, *Cancer Discov* 2021). Given unique driver mutations (i.e. *JAK2*, *CALR*, *MPL*), we hypothesized that the cell surface of MPN-BP would reveal a distinct biological signature and therapeutic targets versus de novo AML. To our knowledge, an MPN BP-specific immunotherapy has yet to be developed. Thus, we took a two-pronged approach: 1) use unbiased proteomics discovery to identify surface proteins enriched in MPN-BP cell lines that have potential for immunotherapy development; and 2) generate immunotherapy against a known target (surface CALR) with specificity to a subset of MPN-BP.

**Methods and Results:** Toward the first strategy, we profiled the cell surface of 5 cell lines derived from patients with transformed MPN: UKE-1 (*JAK2* mutant), HEL 92.1.7 (*JAK2*), SET2 (*JAK2*), MARIMO (*CALR*),MONO-MAC-6, and ELF-153 (both “triple-negative”) and compared to 3 non-MPN AML lines (HL-60,MOLM-13, NOMO-1). We performed mass spectrometry in biological triplicate for each line and identified over 1,000 membrane-spanning proteins using tandem mass tag (TMT) multiplexing. Differential expression analysis identified over 70 membrane proteins enriched in MPN BP-derived cell lines and more than 120 enriched in non-MPN AML lines (Figure 1a). Gene ontology and pathway analysis suggested increased EGFR signaling in MPN, a finding published in prior reports, as well as altered lipid metabolism (Figure 1b). We then focused on C3AR1, a multi-pass G-protein coupled receptor, as a potential therapeutic target given: 1) moderate level of protein expression by proteomics, confirmed by flow cytometry (not shown); 2) enrichment in MPN-derived cells (Figure 1a);and 3) limited mRNA expression in monocytic and granulocytic cells and no significant mRNA expression in other normal tissues or endothelium (per GTEx, BloodSpot and Human Protein Atlas databases, not shown). Flow cytometry on purified CD34+ cells from marrow donors confirmed no C3AR1 expression (not shown).

Toward the second strategy, as proof-of-principle for immunotherapeutic targeting, from the literature we obtained single chain variable fragment (scFv) sequences specific for CALR (targeting a linear peptide of the N terminal domain) and C3AR1 (clone 3G7 targeting the second extracellular loop) and expressed these in a chimeric antigen receptor (CAR) backbone (IgG4 hinge with CD28 transmembrane and co-stimulatory domains). *In vitro* cytotoxicity assays in MARIMO cells engineered to overexpress GPI-anchored calreticulin at the cell surface showed potent activity of the anti-CALR CAR (~100%activity at 2:1 effector to tumor cell ratio in overnight co-culture) but modest activity of the anti-C3AR1CAR (~70% lysis at a 5:1 effector to tumor cell ratio). Consistent with *in vitro* data, a xenotransplantation study in NSG mice showed survival benefit with anti-calreticulin CAR but lesser potency of the C3AR1 CAR (not shown).

**Conclusions**

To our knowledge, the “surfaceome” of MPN-BP cells has not been previously profiled at the depth described here, making this dataset a unique resource to the MPN research community. These data also highlight the potential utility profiling the cell surface of MPN-BP patient samples, which may reveal additional biology or targets not captured in cell lines. While anti-calreticulin and anti-C3AR1therapies represent promising treatment modalities for transformed MPN-BP, further work is required to increase potency *in vivo*. Validating surface levels of calreticulin and C3AR1 in MPN-BP patient samples, and healthy tissues, is now being pursued toward further pre-clinical development.



Harold Amos Medical Faculty Development Program

Name: Simpa S. Salami, MD, MPH

Current Institution: University of Michigan

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College: University of Ibadan, Nigeria

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Title of Project: Molecular Dissection of Clear Cell Renal Cell Carcinoma (ccRCC)

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**ABSTRACT**

**Objective:**There is a critical need to develop prognostic biomarkers to improve the management of patients with clear cell renal cell carcinoma (ccRCC).This study aims to develop and validate gene expression-based biomarkers associated with recurrent disease to facilitate risk-stratification of ccRCC.

**Methods:** We retrospectively identified 110 patients who underwent radical nephrectomy for localized ccRCC. Patients who recurred were matched based on grade/stage to patients without recurrence. RNA next-generation sequencing (NGS) was performed on formalin-fixed paraffin-embedded (FFPE) tissue using whole-transcriptome sequencing on the illumina platform. We developed a gene signature to predict recurrence/progression-free survival (PFS) using a 15-fold lasso and elastic-net regularized linear Cox model. We derived Myriad Prolaris™ commercially available 31-gene cell cycle proliferation (mxCCP) score using RNA-seq data for each patient. Validation datasets were assembled: dataset #1 - The Cancer Genome Atlas ccRCC (TCGA, n= 382) and dataset #2 [Seishi Ogawa Japanese ccRCC (n=87), International Cancer Genome Consortium ccRCC (ICGC; n=81), GSE22541 ccRCC (n=20) and Clinical Proteomics Tumor Analysis Consortium ccRCC (CPTAC; n=91)]. Kaplan-Meier (KM) curves and multivariate Cox proportional hazard testing were then used to validate the independent prognostic impact of the gene signature on PFS and disease specific survival (DSS).

**Results:** After quality control, the training cohort comprised 50 patients with recurrence and 41 patients without, with a median follow-up 26 and 36 months, respectively. There were no significant differences between age, sex, grade, and stage between groups (all p > 0.05). We developed a 15-gene signature which was the only variable independently associated with worse PFS and DSS (PFS: HR=11.08, CI=4.9-25.1; DSS: HR=9.67, CI=3.4-27.7), adjusting for clinical-pathologic variables and mxCCP score. The 15-gene signature was also independently associated with worse PFS and DSS in both validation datasets [Validation #1 (n=382), PFS: HR=2.6, CI=1.6-4.3; DSS: HR=3; CI=1.4-6.1 and Validation #2 (n=279), PFS: HR=1.6, CI=0.7-3.6; DSS: HR=3.1; CI=1.5-6.4] adjusting for clinical-pathologic variables and mxCCP score.

**Conclusion:** We developed and validated a novel 15-gene prognostic signature to improve risk stratification of patients with ccRCC. This signature has the potential to facilitate optimal treatment allocation and may lead to the development of novel therapeutic targets.

Harold Amos Medical Faculty Development Program

Name: Tomeka Suber, MD, PhD

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Title of Project: The role of itaconate in host defense and resilience in intrapulmonary

Klebsiella pneumoniae infection

**Abstract:**

**Project summary:** Gram negative bacteria are the most common pathogens implicated

in nosocomial pneumonia in critically ill patients. Klebsiella pneumoniae (KP) in particular has grown in prominence worldwide with increasing prevalence of antibiotic resistance, hypervirulent strains, and invasive clinical syndromes. Immune mechanisms of host defense responsible for clearance of KP infection from the lung are largely unknown. Cis-aconitate decarboxylase 1 (ACOD1) is a mitochondrial enzyme robustly induced in human alveolar macrophages that catalyzes the production of itaconate. Itaconate has anti-inflammatory effects on macrophages, and ACOD1 polymorphisms disproportionately expressed in people of African descent are associated with increased itaconate production. The goal of this proposal is to define the role of ACOD1 in host defense in the lung during KP infection. This work will elucidate novel immune mechanisms that may be exploited to reduce mortality associated with this pathogen.

**Aim 1:** To examine Acod1-dependent host resilience during acute intrapulmonary KP infection in vivo.

**Aim 2:** To determine if itaconate is host-protective by increasing the cellular integrated stress response (ISR) during KP infection.

**Progress updates:** For **Aim 1**, we have conducted bulk RNA sequencing and metabolomics studies focusing on the transcriptomic signatures in lung, liver, and spleen tissues in wild-type (WT) and *Acod1-/-* mice at time 0 h and 48 h after intrapulmonary KP infection. Transcriptomic signatures in lung show marked suppression of the unfolded protein response (UPR) pathway, a branch of the integrated stress response (ISR), in *Acod1-/-* mice. While there were no differences between groups in targeted metabolomics studies, analyses are ongoing on the large untargeted dataset. **Aim 2** proposes in vitro studies in macrophages and precision-cut lung slices (PCLS) to examine lung tissue dynamics during KP-induced inflammatory changes driven by itaconate. Preliminary studies over the past year confirm blunting of the UPR when itaconate is absent during LPS stimulation. We are developing assays to detect activation of the UPR using a fluorescent dye and to determine how itaconate regulates expression of Bip, IRE1α, and other key target of the UPR pathway. Finally, serum itaconate levels were examined in a cohort of mechanically ventilated patients with acute respiratory distress syndrome (ARDS). Itaconate levels did not differ between airway controls and ARDS patients. However, targeted and untargeted metabolomics studies identified clear signatures associated with ARDS subphenotypes and poor outcomes including vasopressor use and mortality.

**Future Directions:** In summary, itaconate is a critical host metabolite that directs the host response against Klebsiella pneumonia and appears to promote the unfolded protein response within the lung during infection. Integration of temporal and tissue-specific RNA-Seq and metabolomics datasets will identify itaconate-dependent and independent host response pathways during KP infection. The next year is focused on optimizing in vitro assays (microscopy, flow cytometry, and Image Stream) for functional analysis of UPR activation. We are also working to establish the PCLS system, first in murine lung tissue followed by human tissue, with mentors and collaborators at the University of Pittsburgh. These studies will characterize itaconate-dependent ISR mechanisms during the early host response to infection. More importantly, development of these systems will allow for pivots to other metabolic pathways that regulate the UPR in models of acute lung injury and bacterial pneumonia. These long-term studies will be anchored by the serum metabolomics data already acquired from ARDS patients.

Harold Amos Medical Faculty Development Program

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Title of Project: Predictors of Clonal Hematopoiesis and Risk of Hematologic Malignancy

Premalignant expansions of cells bearing cancer-associated somatic mutations become widespread with age. A critical goal of cancer early detection is to identify individuals with pre-malignant states at greatest risk of progression. Clonal hematopoiesis (CH), a premalignant expansion of hematopoietic stem cells possessing one or more somatic mutations in leukemia-associated driver genes. Under the broader category of CH, two conditions are formally defined. Clonal hematopoiesis of indeterminate potential (CHIP) is categorized by CH with somatic mutations detectable at a variant allele fraction (VAF) of ≥ 2% in the absence of a diagnosed blood disorder or cytopenia. Clonal cytopenia of undetermined significance (CCUS) describes CH with somatic variants at VAF ≥ 2% in the presence of unexplained, persistent cytopenias. CHIP/CCUS diagnoses are being made with increasing frequency and it is estimated that >10% of individuals over age 60 have these premalignant conditions. The risk of progression to overt myeloid malignancy in CHIP/CCUS is highly variable and no risk stratification systems currently exist. To address this need, we have undertaken systematic integrated analysis of human genomic data with deep phenotyping guided by three specific aims: **Aim 1)** to define a phenotype of high-risk CHIP/CCUS; **Aim 2)** to determine the genetics of high-risk CHIP/CCUS and **Aim 3)** to derive prognostic models for CHIP/CCUS for clinical and research use. To date, we have utilized a combination of SEER-Medicare data and local clinical data to describe a putative phenotype for high-risk CHIP/CCUS which we observed as a specific pattern of age-related inflammatory diseases that commonly precede diagnosis of myeloid malignancies (Aim 1). In parallel, we have analyzed whole exomes of over 450,000 individuals in the UK Biobank to determine genetic lesions associated with the highest and lowest risk of progression to incident myeloid malignancy in individuals with CHIP/CCUS (Aim 2). Lastly, using a combination of recursive partitioning analysis and multivariable models, we determined laboratory and genetic variables most predictive of incident myeloid malignancy in CHIP/CCUS cases and have combined them into a novel prognostic model for CHIP/CCUS (Aim 3). This predictive model defined three risk strata for CHIP/CCUS and has been validated in both the UK Biobank and two independent patient cohorts (Harrel’s c-index >0.7). This work delivers a simple prognostic framework for CHIP/CCUS, distinguishing high risk minority from the majority of CHIP/CCUS which has minimal risk for progression to overt myeloid malignancy. While we have addressed the clinical and research need for prognostication for individuals known to have CHIP/CCUS, it also shows that most CHIP/CCUS diagnosed is low-risk. Early detection (screening) efforts will have greatest public health impact when focused on the highest risk populations. Future work, by modeling factors which predict the likelihood of having high-risk CHIP/CCUS, will answer the question: “who should undergo sequencing to screen for myeloid malignancy precursors?”