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: 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: 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.

**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.