Dana-Farber/Harvard Cancer Center

CURE

Continuing Umbrella of Research Experiences

Scientific Presentations
Summer 2017

Dana-Farber/Harvard Cancer Center
Continuing Umbrella of Research Experiences (CURE)

Launched in 2002, the Continuing Umbrella of Research Experiences (CURE) Program at Dana-Farber/Harvard Cancer Center (DF/HCC) is an important building block in research training initiatives. Under the direction of the DF/HCC Initiative to Eliminate Cancer Disparities (IECD), this program is designed to provide underrepresented minority high school and college students with a stimulating and rewarding hands-on research experience that encourages students to pursue education and training in the biomedical sciences and careers in basic, clinical, nursing, and population science cancer research.

Dana-Farber/Harvard Cancer Center
Initiative to Eliminate Cancer Disparities (IECD)

The IECD provides a centralized framework and coordinated structure for addressing cancer disparities. It was among the nation's first integrated, inter-institutional, multi-pronged approach for addressing cancer inequities. Its mission is to support and encourage disparities research in all disciplines and across all DF/HCC member institutions. The IECD focuses on five key areas: a) Community engagement and education, b) Reducing barriers to care, c) Facilitating minority participation in clinical trials, d) Fostering diversity in cancer researchers, and e) Enhancing cultural competency

For more information about the IECD or CURE program, contact:

Karen Burns White, Deputy Associate Director
Initiative to Eliminate Cancer Disparities
P: (617) 632-3244, E: karen_burnswhite@dfci.harvard.edu

Emily McMains, PhD, CURE Research Training Coordinator
P: (617) 632-3028, E: emilya_mcmains@dfci.harvard.edu

CURE Scientific Presentations

Yawkey Building, Room 306
Third Floor Conference Center

Tuesday, August 8, 2017
2:30P.M.-5:00 P.M.

Wednesday, August 9, 2017
2:30P.M.-5:00 P.M.

CURE e-Poster Session

Yawkey Building, Room 306
Third Floor Conference Center

Thursday, August 10, 2017
3:30P.M.-5:00 P.M.

Table of Contents

# Indicates a student presenting a poster

African Exposure to Lycopene and Its Effects on Prostate Cancer

Samuel Amaka

Principal Investigator: Timothy Rebbeck, PhD

Mentors: Caroline Andrews, MSc; Brian Fortier

Dana-Farber Cancer Institute

Men of African descent in the United States have the highest rates of prostate cancer incidence in the world. Other studies have shown that due to limited screening, diagnosis, and cancer registration Black African men appear to have lower rates, but in fact their rates may be just as high as African American men. Research on African men is being conducted to better understand this disparity as African American men share many genetic similarities to Black African men. The literature has also studied factors, such as diet, that may influence prostate cancer risk, but not in African men. Under the Men of African Descent and Carcinoma of the Prostate (MADCaP) Consortium, a retrospective study was conducted with several centers in different African countries. The wide array of variables collected by MADCaP include dietary and nutritional factors. High consumption of tomatoes that contain the carotenoid lycopene has been associated with a lower risk of prostate cancer in some studies. However, studies addressing the effects of diet on prostate cancer have produced contrasting and inconclusive results, but most of these studies haven't focused on the dietary patterns of African countries. We used retrospective data from Korle-Bu Teaching Hospital and 37 Military Hospital in Accra, Ghana that focused on tomato servings as well as the 2014 USDA report involving annual tomato consumption per person. We learned that on average, Ghanaian Men consume more servings of tomatoes annually than the average man in the United States. These data suggest that any relationship between tomato or lycopene exposure and prostate cancer may be different in African men than in US men.

Stress and Methylation of HPA Axis Gene FKBP5 and Human Disease Outcomes: A Systematic Review

Weaam Arman #

Principal Investigator: Alexandra Shields, PhD

Mentors: Nicholas Spence, PhD; Stefania Khoda

Massachusetts General Hospital

Epigenetic phenomena, including DNA methylation, play an important role in understanding cancer outcomes, particularly through modifiable psychosocial exposures operating through the Hypothalamic-Pituitary-Adrenal (HPA) axis. There is a limited body of work on the pathways through which psychosocial exposures may affect the development of cancer via the methylation of FKBP5, a gene in the HPA axis. We conducted a systematic literature review to analyze the association between psychosocial stress and methylation of FKBP5 as well as examine the relationship between FKBP5 methylation and cancer. Targeted searches in PubMed and Embase were conducted to identify relevant English language studies. We identified and included twelve studies in the review that assessed the association between stress and DNA methylation of the FKBP5 gene. Prolonged exposure to glucocorticoids that are a result of psychosocial stress directly affected levels of DNA methylation in an adverse manner. Psychosocial stressors of importance were low socioeconomic status, mental illness, childhood maltreatment and neglect, war trauma, and sexual and physical abuse. Twenty articles were found that assessed the link between DNA methylation of the FKBP5 gene and cancer, with two meeting the inclusion criteria. Of the two articles, an association was found between the FKBP5 gene and prostate cancer. Given that previous work has separately linked modifiable psychosocial exposures to DNA methylation and DNA methylation to cancer, future empirical work should address the link between methylation of the FKBP5 gene and cancer outcomes through psychosocial exposures via the HPA axis.

Finding the Optimal Drug and Concentration to Inhibit the Fanconi Pathway in T-ALL

Dylan Barcelos

Principal Investigator: Alejandro Gutierrez, MD

Mentors: James Degar; Gayle Pouliot, MD/PhD

Boston Children's Hospital

T-cell acute lymphoblastic leukemia (T-ALL) is a subtype of acute lymphoblastic leukemia characterized by an aggressive proliferation of T-Lymphoblasts. Targeted exome sequencing on a cohort of 40 pediatric T-ALL patients revealed heterozygous mutations or deletions in genes involved in the Fanconi-BRCA pathway in 38% of cases. The Fanconi-BRCA pathway is a key DNA repair pathway implicated in the repair of DNA interstrand crosslinks (ICLs). ICLs covalently bond to both strands of DNA, obstructing replication and transcription leading to replication stress and single stranded DNA (ssDNA). ATR and other kinases are recruited to ssDNA and activate the Fanconi-BRCA pathway for repair through homologous recombination. It has been shown that heterozygous mutations impair the Fanconi-BRCA pathway. Our goal is to find the optimal drug and concentration to induce replication stress in a model of T-ALL that is haplo-insufficient for the Fanconi-BRCA pathway. We hypothesize the inhibition of signaling proteins in DNA repair pathways could expose haplo-insufficiency within the Fanconi-BRCA pathway resulting in increased DNA damage and apoptosis in vitro. To answer this question, we performed a drug screen using various kinase inhibitors on T-ALL cells engineered with CRISPR-Cas9 to have heterozygous frameshift mutations in the BRCA2 gene and assessed viability. The data shows that viability is similar between wild-type and heterozygous cells when treated with ATR and ATM inhibitors and all cells exposed to the Chk1 inhibitor show no viability. Results for the ATP-competitive dual Cdc7/CDK9 inhibitor (PHA-767491) are more promising. The heterozygous cells demonstrate increased sensitivity at 1uM compared to wild-type. This shows a potential therapeutic window may exist for T-ALL patients harboring heterozygous Fanconi-BRCA pathway mutations using CDK inhibitors and suggests that further study should be done to understand the molecular link between CDK inhibitors and the Fanconi-BRCA pathway.

Distinct Immune Checkpoint Profiles Associated with Molecular and Histological Subtypes in Breast Cancer

Makena Joy Binker Cosen #

Principal Investigator: Aedin Culhane, PhD

Mentors: Aedin Culhane, PhD; Azfar Basunia; Ricardo De Matos Simoes, PhD

Dana-Farber Cancer Institute

One of the most significant recent advances in cancer therapy has been the advent of immune checkpoint blockade inhibitors. Clinical trials exhibit promising results for enhancement of antitumor immunity with the potential to produce durable clinical responses even in advanced and metastatic stages. Despite the great potential, only subsets of patients have responded desirably, with objective response rates between 10-75%. The biological reasons for the heterogeneity in response are poorly understood. Currently there are clinical trials in breast cancer (BC) targeting cell surface receptors CTLA4 (n=3) and PDL1 (n=20), most of which recruit triple negative BC patients. In the present study, we investigate the expression profiles and the respective clinical significance of PDL1, PDL2, CTLA4 and related B7 family proteins in BC with respect to known molecular and histological subtypes. We re-curated the gene, clinical and molecular subtype annotation of tumor samples associated with gene expression in 33 studies with over 3,000 patients. All analyses were performed in R, from which R scripts and software were generated for reproducible research. From this, we extracted a subset of 1439 patients of which 1205 had invasive ductal carcinoma (IDC) and 234 had invasive lobular carcinoma (ILC) in 11 studies. Strong downregulation of the gene E-Cadherin (CDH1) is a known hallmark of ILC but not IDC. To confirm pathologies, we analyzed the expression of CDH1 in each dataset by generating boxplots and histograms. Through this process, we identified possible errors in the data and restricted further analysis to the subset of studies that met our criteria. After curation, we examined the profile of hallmark genesets, gene expression of selected genes, and their clinically significance in each study. We observed distinct immune checkpoint profiles associated with each subtype, which may provide biomarker support for ongoing and new studies of immune checkpoint blockades in BC.

The Role of CCR8 Chemokine System in Melanoma

Kassandra Boada #

Principal Investigator: Andrew Luster MD, PhD

Mentor: Melvyn Chow, PhD

Massachusetts General Hospital

The recent advances in understanding the molecular pathways underlying melanomagensis and the regulation of the endogenous immune response to tumors has led to the development of novel therapies, including targeted therapy and immune checkpoint blockade therapy. Despite promising outcomes, these therapies are still in need of major improvements. It is now clear that effector cells, including CD8+ T cells and NK cells, within tumors have great potential to protect the host from tumor progression. However, many immunosuppressive mechanisms operate in tumors to counteract anti-tumor immune responses. A better understanding of the molecules that control the complex interactions of immune cells in the tumor microenvironment is required for the development of more effective treatment strategies. Chemokines are chemotactic cytokines that orchestrate the migratory behavior and cellular interactions of leukocytes, therefore exert great impact on anti-tumor immune responses. We have data that melanoma tumor growth is retarded in CCR8-deficient mice. The reduced tumor burden observed in these mice is strongly associated with an increased ratio of CD8+ T cells relative to regulatory T cells (Treg), as well as decreased numbers of granulocytic myeloid-derived suppressor cells (MDSC) within the tumor. We hypothesize that the CCR8 chemokine system promotes the establishment of an immunosuppressive tumor microenvironment by recruiting or inducing the expansion of immunosuppressive cells, such as Treg and MDSC, thereby supporting melanoma tumor growth.

The Use of fMRI in Mapping the Motor and Sensory Cortices

Joanne Charles

Principal Investigator: Alexandra Golby, MD

Mentor: Laura Rigolo, MA

Brigham and Women's Hospital

Functional Magnetic Resonance Imaging (fMRI) is a non-invasive method used to image function in the brain. It utilizes Blood Oxygenation Level Dependent (BOLD) imaging, in which areas of the brain that are stimulated have an increased ratio of oxygenated to deoxygenated hemoglobin. The fMRI can distinguish the different levels of blood in the active area due to the difference in magnetic properties in oxygenated and deoxygenated blood. fMRI is used to help guide neurosurgeons in avoiding the eloquent areas of the brain when removing a tumor. The purpose of this research is to understand the ability of fMRI to distinguish the sensory and motor cortex of the brain through a case study subject. The subject is a 49-year-old male who presented with focal seizures in his right lower extremity and spatial agnosia. An MRI revealed a medial mass extending to the motor cortex. A biopsy revealed that the mass was glioblastoma. A clinical fMRI was done with the subject doing a motor task involving left and right hand clenching and sensory stimulation to the left hand. Analysis of the subject's fMRI activation was done through BrainEx software. The spatial relationship between the sensory and motor function was analyzed. The motor activation was 4.7 mm from the lesion and the sensory cortex had 13 mm of activation that was not revealed by the motor task. The two tasks overlapped, but there was more sensory activation revealed by the sensory task compared to the motor task. Post-op notes were taken after the surgery and revealed that the subject's left hand was slower to respond. The subject reports that the problem has improved as time has gone by. The six months post-op notes are pending. These results indicate that an additional sensory task would be useful for surgical planning.

Analyzing Variability in NF-kB Signaling Pathway in HeLa Cells

Neika Christalin #

Principal Investigator: Suzanne Gaudet, PhD

Mentors: Shibin Mathew, PhD; Amy Thurber, PhD

Dana-Farber Cancer Institute

Tumor necrosis factor (TNF) is an extracellular stimulus for NF-kB. In response to TNF, NF-kB activation leads to the transcription of several anti-apoptotic and pro-inflammatory genes. Abnormal activation of this pathway has been associated with several cancers. Cell-to-cell variability is critical in cellular responses and cancer when malignant cells respond differently to treatments. To characterize the variability in the behavior of this pathway, we generated two reporter constructs, one expressing a fluorescently tagged subunit of NF-kB protein (as a readout of signaling dynamics) and another expressing a fluorescent protein under NF-kB binding (as a readout of NF-kB dependent transcription). We transiently transfected HeLa cells with these plasmids, then tracked the expression of the fluorescent proteins after TNF stimulation. Using computational image analysis software (Cell Profiler and ImageJ), we created a pipeline to segment the images of the HeLa cells, tracking the cellular movement and changes in nuclear NF-kB intensity and transcriptional reporter activity over time. Using this workflow, we were able to characterize variability in NF-kB translocation and corresponding transcriptional reporter activity. The workflow developed in this project will be useful for the understanding of cancer progression and the development of cancer therapies and treatments.

CRISPR-a Gene Overexpression Models in Drosophila Melanogaster

Senait Efrem

Principal Investigators: Norbert Perrimon, PhD

Mentor: Jonathan Zirin, PhD

Harvard Medical School

Drosophila have been used extensively to model human diseases associated with reduction or loss of gene function. By contrast, diseases associated with gene amplification, micro-duplication, or other changes resulting in over-expression have been more challenging to model. Over-expression models would be extremely valuable, as they would provide a system for relatively rapid identification of genetic or chemical modifiers of associated phenotypes. Our goal is to develop and validate gene overexpression models in flies using the CRISPR/Cas9 activation (CRISPR-a) system. We screened 104 genes encoding conserved rate-limiting enzymes as our test set. Fly stocks expressing sgRNAs targeted upstream of the transcription start site of these genes were crossed to flies expressing a catalytically inactive form of Cas9 fused to a transcriptional activator (dCas9-VPR). Progeny were evaluated for overexpression phenotypes and a subset of genes were tested further in tissue-specific contexts. We also generated a fly stock which allows for CRISPR-a enhancer/suppressor screening in flies doubly mutant for the tumor suppressor genes PTEN and Rb. Lastly, select genes were screened for enhancement/suppression of PTEN and Rb. Our results indicate that CRISPR-a is an effective and versatile method for generating overexpression phenotypes in flies

Determining Composition of Grain Mixtures Using High Resolution Magic Angle Spinning Magnetic Resonance Spectroscopy

Luther Evans

Principal Investigator: Dr. Leo Cheng, PhD

Mentor: Lindsey Vandergrift

Massachusetts General Hospital

Our lab uses High Resolution Magic Angle Spinning (HRMAS) Magnetic Resonance Spectroscopy (MRS) to examine metabolites in tissue to develop different cancer diagnostic methods. However, HRMAS MRS can also allow us to determine grain composition in a simple fashion, which is a challenge for manufactures purchasing and selling grain mixtures to each other. This technique, unlike mass spectrometry or infrared spectroscopy, can measure metabolites without chemical extraction. Our objective is twofold: (1) develop a simpler method for identification of grain mixture composition and (2) test the analytical model from this project for use in cancer research. The United States Department of Agriculture (USDA) provided 9 grains (flax, corn, sorghum, wheat, millet, rye, oat, rice, and barley) and 5 mixtures.

To validate measurements between researchers, we scanned a test mixture (millet, spelt, rye, corn) for three trials. Each grain (2.5mg) was placed into a rotor and measured using a Bruker AVANCE spectrometer operating at 600 MHz with 3600 rpm spin rate at 25oC. We expect to see similar results between the spectra, which would indicate high reproducibility between researchers and samples. After the initial test, we began scanning the pure 9 grains. Thus far, we have conducted two measurements each for flax, corn, and sorghum. The project will continue by measuring the remaining pure grains and mixtures followed by analysis using an overdetermined linear regression model. This model allows us to predict the composition of the grain mixture. We want to extend this method to cancer research. This is plausible because the mixture of pathological features in tissue samples are similar to a mixture of grains. These pathological features contribute differently to measured metabolite levels. We will apply the analytical model to calculate, and then mathematically remove, these contributions so we can better compare tissues to identify evidence of cancer itself.

Association of an Obesity Genetic Risk Score with Prostate Cancer Risk and Survival

Brittney Gedeon

Principle Investigator: Kathryn Penney, ScD

Mentors: Kathryn Penney, ScD; Maxine Chen, ScD

Brigham and Women's Hospital

Prostate cancer (PCa) is one of the most significant diseases affecting men worldwide, with over 160,000 men diagnosed every year in the United States. BMI has been associated with aggressive prostate cancer, but results of recent studies are conflicting. Analyzing the association of genetic predictors of BMI with PCa may help us better understand the mechanism behind this disease. We set out to discover if a genetic risk score (GRS), created from variants associated with BMI, is associated with prostate cancer risk and survival. A GRS is the most efficient way to aggregate numerous single nucleotide polymorphisms (SNPs) to investigate an association. In a literature review, 97 novel SNPs associated with BMI were identified and were utilized to create a GRS. We examined these associations using genetic and disease data from 1805 PCa cases and 1304 controls from the Physicians' Health Study and the Health Professionals Follow-up Study cohorts. Using the GRS, a linear and logistic regression were run in the statistical programming software R to investigate the association between the GRS and BMI and the GRS and PCa. Next, a survival analysis was conducted using the Cox proportional-hazards model. The association between the GRS and BMI had an unexpected negative association, showing that as the GRS increases, BMI decreases along with it. However, there was a nonsignificant association between the GRS and the risk of PCa (OR=1.20 95% CI: 0.19-1.56, p-value: 0.34) and survival of PCa (HR=0.82 95% CI: 0.65-1.05, p-value: 0.11). In conclusion, these findings suggest that the genetic component of BMI may have no association with the risk and progression of PCa. Further analysis of the GRS and its associated SNPs needs to be conducted to firmly conclude that there is no correlation between the genetics of BMI and PCa.

Uncovering the Functional Significance of Splicing in Gene Regulatory Networks: Building an Isoform-Resolved Network of Transcription Factor Isoform Interactions

Maria F. Heredia

Principal Investigator: David Hill, PhD

Mentor: Gloria Sheynkman, PhD

Dana-Farber Cancer Institute

Although alternative splicing is known to diversify gene expression, there is still very limited information regarding the functional significance of these isoforms produced within the human proteome. Protein isoforms are alternatively spliced variants derived from the same gene that may differ in function. Using network biology, we are able to construct unbiased biophysical profiles that are used to compare the functional differences between protein isoforms. We are investigating a specific class of proteins, transcription factors (TFs), and how splicing alters their functional properties in the context of gene regulatory networks.

Using a high-throughput, binary protein-protein interaction platform, I conducted several screens to discover and quantify the degree of protein interaction between TFs and their protein interaction partners. Specifically, we employed a high-throughput version of the Yeast-2-Hybrid (Y2H) assay where we can measure the biophysical interactions between proteins in a systematic manner. I independently carried out several steps to accomplish this: using liquid robotics to pool yeast strains containing the protein preys to mate (i.e. test) against strains with the protein bait, mating the haploid yeast cells, spotting the cells on selective solid media, and picking the colonies for sequencing. This work resulted in discovery of over 2,190 candidate biophysical interactions and will be used to create isoform functional profiles. Mapping these physical protein interactions will give insight into how different isoform transcription factors may operate differently within the gene regulatory system, which will aid in creating and understanding human disease networks.

The Role of PAD4 in Necroptosis

Carolyn Huang #

Principal Investigator: Ben Croker, PhD

Mentor: Mary Speir, PhD

Boston Children's Hospital

Peptidyl arginine deiminase 4 (PAD4) is an intracellular enzyme that converts arginine to citrulline residues on varied substrates. It is suggested to contribute to the generation of neutrophil extracellular traps (NETs) by promoting the citrullination of histone H3. NETs are composed of webs of chromatin and proteases that can trap and kill microbes. This study examines the role of regulated non-apoptotic mixed lineage kinase domain-like (MLKL)-dependent cell death, namely necroptosis, in NET formation, and the role of PAD4 in this pathway. Necroptosis is characterized by the release of cellular contents and the induction of potent inflammatory responses. Previous work in our laboratory indicated that PAD4 is activated by MLKL and is essential for NET formation. This study further investigated this novel effector function of MLKL-mediated necroptosis in mouse and human neutrophils. Neutrophils isolated from the bone marrow of C57BL/6J mice were treated with chemical inhibitors of PAD4 in the presence or absence of necroptotic stimuli. The levels of citrullinated H3 were evaluated by Western blot and the levels of tumor necrosis factor alpha (TNF) were assessed by ELISA. In mouse neutrophils, the PAD4 inhibitors, GSK484 and GSK199, did not inhibit citrullination of H3. In contrast, the irreversible pan-PAD inhibitor, BB-Cl-Amidine, inhibited H3 citrullination in mouse neutrophils and reduced the production of TNF in vitro. The reduction of TNF was consistent with previous studies that showed direct citrullination of p65 (NF- B) by PAD4 and the associated increase in NF- B-dependent TNF production. The results from this study establish BB-Cl-Amidine as an effective inhibitor of PAD4-dependent functions in neutrophils and pave the way for further investigation into its role in necroptotic cell death.

Possible Involvement of Gasdermin Family in Neutrophil Cell Death

Wesley Hudson

Principal Investigator: Hongbo Luo, PhD

Mentors: Hiroto Kambara, PhD; Yan Teng

Boston Children's Hospital

Pyroptosis is a programmed form of lytic cell death involving the formation of pores on the plasma membrane and the eventual swelling and rupture of the cell, releasing pro-inflammatory cytokines into the extracellular fluid. Recently, several groups reported that a N-terminal fragment of Gasdermin D (GSDMD) cleaved by activated inflammatory caspases triggered pyroptosis. Our lab investigates the mechanisms and pathways controlling neutrophil cell functions. Neutrophils have a short life-span and constitutively die. The molecular mechanism of this "constitutive neutrophil death" remains unknown. Knowing GSDMD's ability to induce pyroptotic cell death, we sought to investigate the involvement of the pyroptosis mediating protein family in neutrophil cell death. The objective of this study was to determine if neutrophils had the ability to cleave GSDMD and induce pyroptotic cell death and identify the enzyme responsible for doing so. Our immunoblotting data showed two GSDMD bands during the course of neutrophil death, suggesting the cleavage of GSDMD. To rule out the possibility of alternatively spliced mRNAs, we performed reverse transcription (RT) PCR. We found no PCR product corresponding to the cleaved GSDMD size, suggesting that there is no alternative splicing during neutrophil spontaneous death. In order to deduce which enzyme cleaved the protein, we treated the neutrophils with a variety of enzyme inhibitors, however, none of them affected possible GSDMD cleavage. We conclude that GSDMD is being cleaved by an unknown enzyme in the neutrophils to mediate cell death. In the future, we plan to optimize enzyme inhibitor conditions to find more conclusive data. Our insight into this process could potentially allow us to discover a new therapy target to help with chronic inflammatory issues.

Identifying Molecular Vulnerabilities in CALR-mutated MPN cells

Jennifer Ihedioha #

Principal Investigator: Ann Mullally, MD

Mentors: Shannon Elf, PhD; Azucena Rocha

Brigham and Women's Hospital

Myeloproliferative neoplasms (MPN) are bone marrow diseases that cause red blood cell, white blood cell, and platelet overproduction. For our project, we focused on identifying the molecular vulnerabilities in MPN cells transformed by mutant calreticulin (CALR), the second most common mutation in MPN patients. CALR is an endoplasmic reticulum protein that regulates ER protein folding and ensures no misfolded protein accumulation. Mutant CALR leads to platelet overproduction in the bone marrow, causing spontaneous clotting. It is thus important to identify molecular vulnerabilities in CALR-mutated cells to improve treatment of CALR-mutated MPN and prolong patient survival. We began by treating CALR wild-type and CALR mutant-expressing cells with various pathway inhibitors previously found to be differentially regulated in mutant CALR versus WT CALR cells. The inhibitors used were 2-DG, a glycolysis inhibitor, oligomycin, an oxidative phosphorylation (OXPHOS) inhibitor, and bortezomib, a proteasome inhibitor. We found that 2-DG treatment had no differential effect on WT versus mutant CALR-expressing cells, suggesting that mutant CALR-transformed cells do not require glycolysis to survive. Our oligomycin and bortezomib treatments are underway. We expect mutant CALR cells to be sensitive to oligomycin. Since they were unaffected by glycolysis inhibition, these cells likely rely on OXPHOS to survive. Likewise, we expect mutant CALR cells to be sensitive to bortezomib since previous experiments in the lab have shown that mutant CALR up-regulates proteasomal degradation. Western blots are being performed to validate unique binding partners to mutant CALR identified in an unbiased proteomics study performed in the lab last year. I expect to validate several proteins that bind only to mutant CALR and not WT CALR. Future studies in the lab will determine the function of these novel binding partners and whether mutant CALR-mediated cell survival is dependent on the interaction between these proteins and mutant CALR.

Men of African Descent and Cancer of the Prostate (MADCaP) Project

Regine Jean-Baptiste #

Principal Investigator: Timothy Rebbeck, PhD

Mentors: Caroline Andrews, MSc; Brian Fortier

Dana-Farber Cancer Institute

Prostate cancer is the most common cancer among men worldwide and it is among the leading causes of cancer deaths. Well-established risk factors include age, ethnicity and family history of the disease. Genetic predisposition may be due to low penetrance genetic variants. Nutrition-related factors such as obesity and physical activity play an important role in the occurrence of several cancers but the evidence for obesity, physical activity, or specific foods and nutrients in prostate cancer risk is less consistent. We examined several meta-analyses which evaluated the possible association between prostate cancer, diet, nutrition, obesity and physical activity in US men. Only 21% of the examined meta-analyses gave a nominally statistically significant finding based on the random effects model. This percentage diminished dramatically when stronger P-value thresholds were considered. Men of African descent have the highest incidence of prostate cancer in any racial or ethnic group, and they have the highest rate of aggressive disease and mortality. No obesity studies have previously been undertaken in African men. We analyzed height and weight data obtained from the Men of African Descent and Cancer of the Prostate (MADCaP) Project from 294 men from two tribes in Ghana and compared this with the occurrence of prostate cancer in men from the US. Our analyses suggest that height and weight are higher in the Ghanaian cohort than in the USA. If these variables are associated with prostate cancer risk, the higher rate of prostate cancer may be influenced by these variables in Ghana to a greater degree than in the US.

ABO Blood Group Typing of Stem Cell Transplant Donors Using Next Generation Sequencing

Abigail Joseph #

Principal Investigator: William Lane MD, PhD

Mentor: Helen Mah, MS

Brigham and Women's Hospital

ABO is the most clinically significant blood group; correct antigen determination is essential for matching patient and donor for transplants. Clinical ABO typing uses serological reagents that type for A, B, and O antigens but lack the sensitivity to discriminate among A and B subtyping, weak expression, or hybrid phenotypes. Although ABO types can be predicted with Sanger sequencing and DNA SNP assays, these tests are not reliable enough for clinical testing. Without a reliable molecular test, Stem Cell (SC) transplant donor registries are unable to acquire ABO typing data from donor registrant buccal swabs, which hinders the allocation of ABO compatible transplants. Forty to fifty percent of leukemia lymphoma patients receive ABO incompatible SC transplants and may suffer from delayed erythroid engraftment and haemolysis of residual red blood cells. We sought to create an improved and more reliable DNA based ABO assay using Next Generation Sequencing (NGS) and companion Computational Interpretative Software. Haplotype cis-trans phasing information was incorporated into CIS, allowing for ABO antigen phenotyping at serologic equivalent levels and ABO A2 subtyping of 110 whole genome sequences. We then created an exon 2-7 targeted NGS assay using 150bp reads to facilitate long-range phasing for typing at a high resolution allele level. We tested 77 samples, of which 17 were rare and difficult to type subtypes with 98.7% concordance with serology. We then created a similar exon 6-7 targeted NGS assay, for use with the more degraded DNA isolated from buccal swab samples, and tested 61 samples with 98.3% concordance with serology. These findings suggest that targeted NGS assays can be comparable in accuracy to serologic procedures. They support the use of these assays to confidently identify weak, rare, or hybrid ABO subtypes and to predict ABO phenotype using DNA isolated from buccal swab samples for transplant registries.

Determining the Enhanced Metastatic Phenotypes Caused by FOXP2 Knockdown in Breast Cancer Cells

Alex Kaminsky

Principal Investigator: Antoine Karnoub, PhD

Mentor: Julie Konge, PhD

Beth Israel Deaconess Medical Center

In the United States, breast cancer accounts for 15% of all new cancer cases and is the second most common type of cancer among women. Patients with localized breast cancer exhibit elevated therapeutic response and survival rates. Difficulty in treating breast cancer, however, arises when the tumor undergoes metastasis, a process through which tumor cells move from primary tissue and form growths in secondary tissues. The majority of deaths from breast cancer are caused by tumors that have undergone metastasis, typically to the brain, bone, and liver. Thus, understanding how cancer cells develop the properties required to metastasize is crucial to the development of therapies that can improve patient survival. Previous research in the lab has identified a major pathway linked to breast cancer progression to metastasis, which involves a transcription factor called Forkhead box protein P2 (FOXP2). It was shown that downregulation of FOXP2 in breast cancer cells promoted tumor initiation in mice and caused the development of prominent lung metastasis. However, how FOXP2 inhibition specifically enhanced metastasis phenotypes in cancer cells was not fully determined. The goal of my research project was to characterize some of the in vitro phenotypes of FOXP2-ablated breast cancer cells and determine the activation levels of some of the signaling pathways that are most commonly associated with these traits. Ultimately, we found that while FOXP2 knockdown had no effect on the ability of isolated breast cancer cells to grow in 2D-attached culture conditions, it caused marked improvement in their ability to grow in anchorage-independent 3D-environments. This suggests that FOXP2 knockdown promotes tumorigenesis and metastasis, in part, by decreasing the anchorage-dependent growth characteristics of cancer cells, providing novel insights into how FOXP2 downregulation enhances breast cancer malignancy.

Use of Kinase Inhibitors to Examine NF2-dependent Autocrine Signaling

Breelyn Karno

Principle Investigator: Vijaya Ramesh, PhD

Mentors: Roberta Beauchamp; Luke Witt

Massachusetts General Hospital

Neurofibromatosis 2 is caused by loss-of-function mutations in the NF2 tumor suppressor gene, often resulting in formation of benign CNS tumors including meningiomas and schwannomas. In addition, loss of NF2 is also found in the sporadic counterparts of these tumors in the general population. However, NF2 protein function remains unclear. NF2 has been shown by the Ramesh lab and others to regulate various pathways including mTOR, MEK/ERK, Hippo/YAP, and EPH Receptor/SRC signaling which have been shown to exhibit cross-talk. Using arachnoidal cells (ACs), the cell type of origin for meningioma, where NF2 has been deleted using CRISPR/Cas9 genome editing, the Ramesh lab demonstrated increased expression and secretion of a ligand of interest which specifically binds a family of receptor tyrosine kinases (RTK), suggesting an autocrine loop in these cells. Previous reports demonstrated that stimulation of this ligand/RTK inhibits downstream Hippo signaling and activates the transcription factor YAP, leading to increased ligand expression, thus forming an autocrine loop. Because of cross-talk, we asked whether inhibitors of these implicated pathways, including Rapamycin and AZD2014 (mTORC1 and mTORC1/2 inhibitors respectively), Trametinib (MEK inhibitor), Verteporfin (YAP inhibitor), and Dasatinib (EPH/SRC inhibitor), would lead to changes in the expression of this ligand. Using quantitative RT-PCR, we found that 24h treatment of NF2-null ACs with mTOR inhibitors AZD2014 and Rapamycin appeared to decrease ligand expression by approximately 50% compared to vehicle-treated control cells, suggesting that ligand expression is partially regulated by mTOR signaling. These results suggest a role for NF2 in regulating ligand/RTK signaling via regulation of mTOR signaling. Next, our lab will complete testing of inhibitors of the remaining pathways of interest to more completely characterize the mechanism of regulation of the implicated ligand. Results from these experiments will give a better mechanistic understanding of the regulation of ligand/RTK signaling by NF2.

Clinical Significance of Reduced Allelic Fractions Among Li-Fraumeni Syndrome Patients

Emmanuel Messele #

Principal Investigator: Judy Garber, MD, MPH

Mentors: Camila Gabriel, MSc, MS; Alexander Husband

Dana-Farber Cancer Institute

Li-Fraumeni syndrome (LFS) is a cancer-related syndrome, typically caused by a mutation in TP53, associated with childhood and adult onset cancers such as soft-tissue sarcoma, osteosarcoma, premenopausal breast cancer, certain brain tumors, adrenocortical carcinoma, and leukemias. The lifetime risk of cancer is 90% amongst women and 73% amongst men. A classic germline mutation is one found in all cells of the body at a rate of approximately 50% allelic fraction. A mutation of somatic cells cannot be passed on genetically and is often only seen in tumor tissue. In those with a germline mosaicism, the mutated DNA is not reflected throughout the tissue and is found at a lower allelic fraction in blood. Here, we have investigated the effects of mosaicism in LFS patients at lower than 40-50% allelic fraction. We hypothesized that if a patient has a mosaic mutation in TP53, then it is expected that, compared to those with classic LFS, there will be lower lifetime risk of cancer and smaller incidence of childhood cancer. We conducted this comparison by juxtaposing patients with 15-20% allelic fraction, a potential germline mosaic mutation, with age and gender matched classic LFS patients with a mutated allelic fraction of 45-50%. We also conducted a comparison of classic LFS patients with an allelic fraction of 45-50% with mutations matched to the patients with 15-20% allelic fraction. We concluded that people with a 15-20% allelic fraction had no family history of LFS but similar cancer incidence, albeit at later ages and no childhood cancer. In addition, these patients did not meet the criteria for LFS. Our next steps will be to expand our cohort of patients, with the long term goal of creating better screening for patients with LFS mosaicisms.

Mechanism of E2F4-Dp1 Removal from DNA in Mid G1 Stage

Mina Milad #

Principal Investigator: James DeCaprio, MD

Mentor: Amy Schade

Dana-Farber Cancer Institute

The DREAM complex (E2F4-DP1, RB-like and MuvB) binds to and represses expression of cell cycle genes during the resting state of the cell cycle called quiescence. The DREAM complex is disrupted during cell cycle entry from quiescence to allow the expression of cell cycle genes necessary to enter S phase. Our lab discovered that p130 and MuvB proteins lose interaction from cell cycle promoters early during G1, but E2F4-DP1 persistently bind cell cycle promoters until mid/late G1 cell cycle. The mechanism of loss of E2F4-DP1 interaction with cell cycle promoters is unknown. E2F1 is a transcriptional activator that binds to cell cycle promoters during mid/late G1 and may contribute to the loss of E2F4 binding. We hypothesize that E2F1 out-competes E2F4-DP1 for binding to cell cycle promoters and Cyclin-CDK phosphorylation reduces E2F4 binding affinity to cell cycle promoters. We found E2F4-DP1 protein expression does not change throughout the cell cycle, suggesting that its binding to cell cycle promoters is what changes. We plan to determine when E2F1 is expressed during the transition from quiescence to S phase. We will complete in vitro binding assays to test if E2F1 binding results in loss of E2F4-DP1 from promoters. I am working to generate plasmids that have DP1 and E2F4 in a bacterial expression vector called pGEX, which will help us express the protein in E. coli BL21 competent bacteria. We have developed a cloning strategy using restriction enzymes to isolate the genes of interest to ligate them into the pGEX vector. I am working to determine the temperature to induce expression of E2F1 in E. coli BL21. By understanding this removal mechanism, we can better understand a significant inhibitory complex that is absent or mutated in many tumor cells.

Engineering Allosterically-Regulated CRISPR-Cas9 Constructs

Ashley Njiru

Principal Investigator: Matthew Ramsey, PhD

Mentor: Yvon Woappi, PhD

Brigham and Women's Hospital

Skin cancer is driven by several genetic lesions found at particular genomic sites. To closely study these aberrances, CRISPR/Cas9 endonuclease systems have been employed. We aim to engineer a lentiviral CRISPR/Cas9 system able to precisely infect and edit murine and human squamous cell carcinoma (SCC) cells. We fused an allosterically-regulated Cas9 complex (arC9) onto a lentiviral backbone and inserted an enhanced Green Fluorescent Protein (EGFP) detectable by FACS, enabling us to attain precise infection and spatiotemporal control of cleaving activity. We used the BamH1 and EcoR1 endonucleases to excise mCherry out of a Lenti-arC9-mCherry construct previously engineered in the lab and designed EGFP primers with BamH1 and EcoR1 restriction sites to incorporate sticky ends onto EGFP amplicons. Amplicons were then ligated onto the Lenti-arC9 construct. The assembled Lenti-arC9-eGFP construct (pLAG) was transformed into E. Coli cells. These colonies were grown onto ampicillin-containing agar plates then picked after 16hrs to be assessed for EGFP incorporation by colony PCR. MaxiPrep was performed to isolate pLAG constructs. Constructs were transfected into HEK293T cells to produce viral packaging components necessary to infect our SCC lines. After confirming EFGP expression in the 293T cells, SCC-13 lines were infected with the pLAG and examined for EGFP expression. Digestion of Lenti-arCas9-mCherry was validated by gel electrophoresis. Colony PCR revealed that most E. coli colonies contained the pLAG construct. Both transfected HEK293T cells and subsequently infected SCC-13 cells showed robust EGFP expression. Our experiments demonstrate that our newly engineered pLAG construct is effective at transfecting HEK293T cells and at infecting mammalian SCC cells. Ongoing experiments are attempting to insert a selective Puromycin marker into the pLAG construct, enabling high-throughput assessment of infection success. Future work will focus on using these constructs to investigate specific genes driving skin neoplasia.

Targeting Neuropilin 2 as an Anti-Cancer Strategy

Ellah Nzikoba

Principal Investigator and Mentor: Diane Bielenberg, PhD

Boston Children's Hospital

Neuropilins (Nrp) are transmembrane receptors expressed in tumor cells and tumor-associated endothelial cells. There are two Neuropilin receptors (Nrp1, Nrp2) that bind to the angiogenic protein, vascular endothelial growth factor (VEGF). Systemic targeting of Nrp with neutralizing antibodies inhibited cancer progression in preclinical trials. In fact, inhibition of Nrp2 using stable Nrp2-shRNA strategies in the tumor cell compartment resulted in smaller pancreatic tumors in mice. Our objective was to determine whether tumor angiogenesis, the growth of new blood vessels into the tumor microenvironment, is dependent on Nrp2 expression. We tested our hypothesis, that tumors require Nrp2 in stromal cells in order to grow in vivo, by injecting syngeneic tumors into wildtype C57Bl6 mice and Nrp2-knockout mice and measuring tumor growth. Luciferase-labeled MB49 bladder carcinoma cells were injected orthotopically into the bladder of mice and tumor growth was monitored via the IVIS Xenogen imaging system. The vascular Nrp2 dependence was compared between several cancers including bladder, breast, pancreas, and melanoma. Our results indicate that melanoma was the most dependent on Nrp2 in tumor-associated vessels, pancreatic cancer was moderately affected, while bladder and breast cancers grew similarly in Nrp2-deficient mice compared to controls. Additionally, we compared the potential for systemic toxicity between targeting Nrp1 versus Nrp2. A Phase 1B human clinical trial reported renal toxicity and proteinuria following treatment with antibodies to VEGF and NRP1, while Nrp2 antibodies have not been tested in humans to date. Our new data indicates that nephrons lack of Nrp2 expression and deletion of Nrp2 in mice did not alter urinary protein levels, suggesting that Nrp2 therapy is non-toxic. Taken together, our data suggests that systemic targeting of Nrp2 may inhibit both tumor cells and endothelial cells.

Evaluating and Improving the Performance of Cancer Risk Prediction Tools

Nofal Ouardaoui #

Principal Investigator: Giovanni Parmigiani, PhD

Mentors: Danielle Braun, PhD; Amanda Blackford, Sc.M.; Cathy Wang

Dana-Farber Cancer Institute

Although many cancer inducing mutations develop through an individual's exposure to external, environmental factors, germline cancer mutations are unique in that they are genetically inherited from parents to offspring. Several statistical models have been developed to estimate both the probability that an individual is a mutation carrier and their future risk of cancer based on family history. One such model is MMRpro, a Mendelian risk prediction model, which estimates the probability that an individual has a mutation in one of the Mismatch Repair genes, more specifically, MLH1, MSH2, and MSH6, and future risk of colorectal and endometrial cancer based on family history. The model uses Mendelian laws of inheritance, mutation prevalence, or frequency of a mutation at a population level, and penetrance, the probability of individual developing a disease at a specific age based on mutation carrier status. The current version of MMRpro uses penetrance estimates from a meta-analysis conducted by Chen et al (2006). The goal of this work is to evaluate the performance of MMRpro using penetrances recently estimated by our group as part of our work on ask2me.org, a risk prediction tool for individuals with genetic mutations. We evaluate the performance of MMRpro on a dataset containing 1032 individuals, from eight clinical sites, whose family history and genetic testing results were collected. After generating mutation carrier risks for each family consultant, we compare the performance of the current version of MMRpro to that of MMRpro with updated penetrance estimates from ask2me.org using measures of mean squared error of prediction (MSEP), area under the response operating characteristics curve (ROC-AUC), and the ratio of observed to expected number of events. This assessment will allow us to analyze the significance of changing penetrance on the model's performance and thus, its ability to effectively guide consultants on their cancer risk.

Systematic Mutagenesis of p53

Sebastian Palacio-Ramirez #

Principal Investigator: William Hahn, MD, PhD

Mentor: Andrew Giacomelli

Dana-Farber Cancer Institute

p53, also known as TP53, is a stress-responsive tumor suppressor gene that can regulate the cell cycle, orchestrate DNA repair, and if necessary, trigger apoptosis. The human p53 gene is the most commonly mutated gene in human cancer, but different mutations in p53 can have different functional consequences. Loss-of-function (LOF) mutations in a single allele can reduce the capacity of the cell to mount a p53 response; if both alleles are mutated or deleted, cells will be totally deprived of tumor suppression by p53. Many cancer-associated missense mutant p53 isoforms have been found to exert dominant–negative (DN) effects on co-expressed wild-type (WT) p53 by forming mixed tetramers that are incapable of DNA binding and transactivation. This means that even if one WT allele is retained, the p53 pathway may be severely compromised, particularly if the mutant protein is expressed in excess over its WT counterpart. However, despite decades of research, a comprehensive model that describes the relative importance of LOF and DN activity in the selection of specific p53 mutations in human tumors is lacking. Thus, we aimed to systematically test every possible mutant p53 variant for LOF and DN activity. Using a high-throughput mutagenesis technique, we made missense and nonsense p53 mutations in a pooled format. We then performed screens in human cancer cells to identify mutant p53 alleles that exhibit WT function, LOF, or DN activity. To validate and extend the findings of this screen, we re-made 70 specific mutant alleles via site-directed mutagenesis. We successfully created 70% of the designed mutations and cloned these into lentiviral expression vectors. Sanger sequencing confirmed that these constructs contained mutations of interest with no other undesired mutations. Introduction of these constructs into human cancer cells will allow us to study the function of these mutant p53 variants in greater detail.

Anti-Malaria Drug as Anti-Cancer Chemotherapeutic in Pediatric Brain Tumors

Darina Paulino #

Principal Investigator: Bahkos Tannous, PhD

Mentor: Jian Teng, PhD

Massachusetts General Hospital

Brain tumors are the most common solid tumors among children under the age of 15 and represent about 20% of all childhood cancers. Pediatric high-grade gliomas (pHGGs; WHO grades III and IV) account for approximately only 7% of the brain tumors but are the main cause of cancer-related death in children, with a 5-year survival rate of 15–35%. We are in need of new therapeutic agents that can help eradicate this disease. We evaluated a subset of bioactive compounds and identified an FDA-approved member of the quinoline class of antimalaria agents, mefloquine, as a promising anticancer chemotherapeutic in pediatric brain tumors. We achieved this by using a high-throughput in vitro drug screen with two chemical libraries and validation. Our overall results suggest that mefloquine induces cell apoptosis through Reactive Oxygen Species (ROS) signaling. An increase in ROS production leads to oxidative stress, which leads to damage in cell structures. This project is focused on the signal transduction pathway involved in cell apoptosis presumably caused by an increase in ROS. In vitro I treated SJ-GBM2, a primary cell culture of pHGGs, with a low concentration of mefloquine (500 nM) for 18 hours. Myc and Ki67 protein are important in the cell apoptosis and cell proliferation process and were detected by western blotting. The anticipated results are that Ki-67 and Myc expression will be significantly inhibited by low dose of mefloquine. In vivo we will be injecting mice with the pHGG cells, treating with low doses of mefloquine and monitoring tumor growth. These results will elucidate whether mefloquine can be used as a therapeutic against pHGGs and potentially lay the groundwork for translation of this compound to the clinic.

Review of Worldwide Cancer Survivorship Guidelines

Robert Pepen

Principal Investigator and Mentor: Larissa Nekhlyudov, M.D., M.P.H.

Brigham and Women's Hospital, Dana-Farber Cancer Institute

Although cancer patients can be 'cured' of their cancer, the long-term effects of treatment (chemotherapy, radiation, and surgery) can lead to various different problems such as another cancer, heart problems, bone problems, and many others. Many patients are left without guidance on how to deal with such long-term effects because it is assumed they are completely healthy after the eradication of their cancer. If primary care providers had guidelines to follow on how to follow up with patients after cancer survivorship, then the possible effects of treatment can be prevented, improved, or treated. There are a number of guidelines available in the United States and abroad, but it is not known where they exist and how similar they may be. In this project, we searched for cancer survivorship guidance/guidelines by using key terms on Google. Links that held the information were then placed into a spreadsheet tab corresponding to the geographical region's country. The regions studied were North America, Europe, the Caribbean, the Middle East, Oceania, Asia, Central and South America, and Africa. Each region contained various countries that were individually researched. After searching for the cancer survivorship guidance/guidelines provided online for each region, we found that North America, Europe, and Australia had more guidance compared to Africa, the Caribbean, and Central and South America. The ultimate goal of this project is to review the content of survivorship guidelines worldwide in order to improve those that are existing and provide input to countries that do not currently have any such information.

Comparison of the Prevalence and Types of Mutations in Prostate Cancer Among Black and White Populations

Hanh Pham

Principal Investigator: Franklin Huang MD, PhD

Mentors: Thomas Randall, PhD; Paz Polak, PhD

Broad Institute, Dana-Farber Cancer Institute, Massachusetts General Hospital

Prostate cancer (PCa) is the second leading cause of cancer death in men. Strikingly, men of African ancestry have the highest rates of incidence and mortality. Whether a genetic basis is behind this disparity is unclear. To establish a molecular taxonomy of the disease for future diagnostic, prognostic, and therapeutic stratification, The Cancer Genome Atlas (TCGA) characterized 333 primary prostate cancers. However, large-scale genomic studies like this predominantly analyze tumors of white patients of European ancestry. To understand relationships between tumoral genomic alterations and ancestry, we characterized cancer genes in 159 black and 404 white PCa patients. We aggregated PCa genomic data from the Broad Institute with TGCA cohort data and analyzed the prevalence and types of mutations among black and white patients. First, we identified significantly mutated genes (SMGs) in each cohort using MutSigCV. The SMGs in the African American (AA) cohort include: SPOP, TP53, KDM6A, ZMYM3, ERF, ZNF131, and ATM; while SMGs in the European ancestry (EA) cohort include: TP53, SPOP, PTEN, PIK3CA, CTNNB1, KDM6A, FOXA1, NTM, APC, BCLAF1, FAM120B, ZFHX3, HRAS, MLLT10, SEC16A, TBXA2R, EHHADH, CR1L, ZNF292, MED12, and MED15. We compared mutational frequencies of these genes between cohorts using Fisher's exact test. While we observed a higher frequency of mutations in TP53 in the EA cohort vs the AA cohort, and in FOXA1 and SPOP in the AA cohort vs the EA cohort, overall mutational frequencies were not significantly different between black and white populations in localized PCa after correction for multiple hypothesis testing. Molecular and genetic profiling is important in subtyping cancers and guiding selection of targeted therapies. While the SMGs in this cohort analysis were not significantly different, differences in copy number and expression require further investigation. To address racial disparities in PCa outcomes, more minorities must be encouraged to participate in genomic studies.

Investigating the Role of F-box in Glioblastoma Multiforme

Philip Phan #

Principal Investigator: Christian Badr, PhD

Mentor: Kelsey Pinkham

Massachusetts General Hospital

Glioblastoma multiforme (GBM) is an aggressive and malignant brain cancer most common in adults. Remission is extremely low as there is generally poor prognosis with less than a 10% 5-year survival rate. GBM can be specified into four subtypes: mesenchymal, classical, proneural, and neural. Mesenchymal and classical often have poorer prognoses when compared to the other subtypes. Our project focused on the presence of genes that affected the malfunctioning of ubiquitin proteasome system (UPS). UPS plays a role in homeostasis by ubiquination and degradation of substrate proteins. However, malfunctioning of the UPS can cause diseases or provide fitness benefits to the tumor cells of GBM. Blocking UPS can prevent tumor growth and lengthen survival rate. Using GlioVis, an online database of brain tumor datasets, 71 F-box genes that are subunits of the structure of the SKP1-CUL1-F-box (SCF) ubiquitin ligase complexes were analyzed. The genes analyzed were then narrowed down based on a significant difference in patient survival rates, unregulated gene expression in classical and mesenchymal subtypes compared to proneural and neural, and prediction of poor prognosis. Expression of 6 FBX genes identified by this analysis can now be investigated in GBM and differentiated GBM cells through quantitative polymerase chain reaction (qPCR).

Development of a Unique Food Composition Database to Identify Novel Biomarkers of Yogurt Intake

Ianick Pires #

Principal Investigator: Eric Rimm, ScD

Mentor: Kerry Ivey, PhD

Harvard T. H. Chan School of Public Health

There is increasing evidence that probiotic bacteria improve gastrointestinal tract health, however, the extent to which probiotic bacteria can change microbiome composition remains unclear. Our limited capacity to assess probiotic strain intake at a population level has hindered progress in this field. As such, we aimed to create a novel probiotic food composition database and explore the association between probiotic intake and microbiome composition. At a species level, we identified all probiotic microorganisms present in commonly consumed US yogurts and applied this to a cohort of 300 adult males to determine their probiotic strain intake. Food frequency questionnaire and 7 day dietary records provide reasonably valid estimates for intakes of a wide variety of dietary variables. We also applied this database to determine the relative abundance of probiotic bacteria in the fecal microbiome. With this model, we observed that in addition to being the most used cultures in yogurts, the most common cultures used by companies and consumed by the cohort were L. acidophilus, L. bulgaricus, and S. thermophilus. We observed that long-term, habitual, frequent yogurt consumption was significantly associated with a higher relative abundance of S. thermophilus (multivariate adjusted $P = 0.009$), confirming our findings that yogurts consumed by US males are a rich source of lactic acid bacteria. These findings were confirmed in analyses using weighted food record data, where the relative abundance of S. thermophilus was significantly different to null in participants who consumed S. thermophilus ($P< 0.001$). These findings indicate potential utility of S. thermophilus as a potential novel biomarker of yogurt intake. Our results also highlight the quality of our dietary assessment and food composition database, which we can now apply to exploring probiotic-microbiome-health relationships.

FDG-PET/CT Measurements of Glucose Metabolism in Healthy Liver

Destiny Porte

Principal Investigator: Annick Van den Abbeele, MD

Mentors: Keisha McCall, PhD; Jiani Hu, MPH; Su-Chun Cheng, ScD

Dana-Farber Cancer Institute

[18F]-fluorodeoxyglucose positron emission tomography/computer tomography imaging (FDG-PET/CT) is used to evaluate glucose metabolism rates in cancer cells and some normal tissues such as the brain and liver. Physicians use FDG-PET/CT images to monitor cancer cells before starting treatment (baseline) and during (follow-up). Standard uptake value (SUL) is used to measure cancer cell metabolism and evaluate response to treatment. Additionally, SUL in normal liver tissue is used to evaluate differences in the equipment or the technique used to acquire the images. The purpose of this project was to test the hypothesis that there are measurable variations in SUL in liver which are independent of equipment or techniques. In a retrospective clinical trial, SUL was measured in the liver of 27 patients who had baseline and one or more follow-up FDG-PET/CT images at a single institution where the same equipment and technique was used for image acquisition. Patient demographics and body habitus was also collected and included in the study data. Study data were grouped into 3-month time intervals relative to the patient's baseline. Variations in SUL between all the patients at each time interval was visually represented using box-plots. The variations between each patient's SUL over the time intervals were illustrated by spaghetti plots. The data was tested for statistical difference between the SUL at baseline and follow-up. Average SUL measurements at baseline for all 27 patients were median 1.7 and interquartile range 0.2. Of the 27 patients, 13 patients had a 3-month follow-up PET/CT, 19 patients had a 6-month follow-up, and 5 patients had both a 3-month and a 6-month follow-up PET/CT. There was no significant difference between SUL at baseline and at 3-month follow-up ($n = 13$ patients, Mann-Whitney U-test $p > 0.05$), and between SUL at baseline and at 6-month follow-up ($n = 19$ patients, Mann-Whitney U-test $p > 0.05$).

Measuring Immune Reconstitution in Anti-PD-1 Lymphoma Patients Post Autologous Stem Cell Transplant

Obed Posada

Principal Investigator: Jerome Ritz, MD

Mentors: Carol Reynolds, PhD; Marie Chammas

Dana-Farber Cancer Institute

We are analyzing a phase II 3 arm protocol designed to determine the clinical effectiveness of adding pembrolizumab, an anti-PD-1 monoclonal antibody, for consolidation therapy after autologous stem cell transplant (ASCT) in patients with relapsed or refractory lymphoma. The three patient cohorts are defined as either A: Diffuse Large B cell non-Hodgkin lymphoma (DLBCL), B: Classical Hodgkin Lymphoma (HL), also of B cell origin, or C: Peripheral T cell lymphomas (PTCLs), a heterogeneous group of lymphomas. The overall study consists of 63 patients to date. We will compare the difference in reconstitution of the immune system in each cohort by analyzing the peripheral blood samples taken at specified time points from each patient. Immunophenotypic analysis is performed on patient samples looking at extracellular surface markers using a four-tube antibody cocktail panel. Tube 1 focuses on T-cell subsets, tube 2 on B-cell subsets, tube 3 on natural killer-cell subsets, and tube 4 on dendritic cell subsets. The samples are run on a Becton Dickenson (BD) LSRFortessa flow cytometry instrument and analyzed by BD FACSDIVA software.

Pembrolizumab binds to the PD-1 receptor(s), inhibiting PD-1 binding to its receptor and interfering with downstream effects of these interactions. This study aims to investigate the anti-tumor activity associated with this check-point blockade activity. To this aim the antibody is administered by intravenous infusion (IV) once every 3 weeks for a total of 24 weeks. Peripheral blood samples are collected at weeks 1, 3, 4, 7, 10, 16, 22, and months 12 and 18 after autologous transplantation. The comparison of data on lymphocyte subset progression throughout these established time points will aid clinicians as they continue to look at this drug for an immune enhancement agent in the autologous transplant setting for lymphoma patients.

Survivin, Exon 16, and DUP Minigene Plasmid Purification

Graciella Rios Ortega #

Principal Investigator: Edward J. Benz, MD

Mentor: Shu-Ching Huang, PhD

Dana-Farber Cancer Institute

Our laboratory studies how mRNA isoform expression is regulated. Alternative splicing is the process by which coded regions called exons are joined together and noncoding segments called introns are removed. The removal of introns and joining of exons depends on the recognition and the usage of 5' and 3' splice sites by splicing machinery. In addition, there are splicing factors, which are proteins that bind to a certain part of the pre-mRNA to induce production of a particular isoform. The balance between positive and negative regulation of splice site selection results in either inclusion or exclusion of an alternative spliced exon. My project is to amplify the provided minigene plasmids Survivin, Exon 16, and DUP for later use to test the effect of splicing factors on alternative exon expression. Exon 16 is a minigene consisting of exon 13, 16, 17 and flanking introns of protein 4.1R. Expression of exon 16 is important for spectrin and actin interaction. DUP minigene consists of exon1, 2, 3 of β-globin and is used for analyzing cis-element for exon 2 expression. Survivin minigene consists of exon 2, 2B, and 3. Survivin will be used to detect exon 2B, whose expression alters the apoptotic nature of the protein. These minigenes were introduced into DH5α bacteria cells and plated on AmpR plates. Colonies were screened using minigene specific primer sets and plasmids were isolated using the Qiagen plasmid isolation kit. Finally, the quality of the plasmids was checked using restriction digestion and the plasids exhibited the correct digestion pattern. These minigenes are ready to be used in co-transfection analyses with several splicing factors (SRp20, SRp40, SRp55, RBM25, SF2, and SC35) whose putative binding sites have been identified on the minigene.

Anti-MiR-21 Therapy Inhibits Angiogenesis in Pancreatic Tumors

Aly Toure

Principal Investigator: Frank Slack, PhD

Mentor: Maud Emmanuel Gilles, PhD

Beth Israel Deaconess Medical Center

MicroRNAs (miRNA) are small non-coding RNA molecules that regulate gene expression post transcriptionally by interaction with messenger RNA (mRNA). Inducing a deregulation in protein levels, miRNAs can influence the growth of many cancers through the modification of oncogene and tumor suppressor gene expression. MiRNA-21 (miR-21) has been found overexpressed in multiple types of cancer and identified as an angiogenesis inducer, creating new blood vessels and increasing the blood flow around tumors in different tumor types. Preliminary studies performed in the lab showed that miR-21 also played a major role in pancreatic cancer tumor progression and that its inhibition impaired pancreatic cancer growth. For the purpose of this project, we decided to study the role of miR-21 in tumor angiogenesis. First, we conducted a phenotypic investigation of tumor sections. We worked with PDAC tumors that had been treated with anti-miR-21 therapy for 30 days (repeated injections). In order to investigate how anti-miR-21 therapy influenced tumor angiogenesis, we performed immunostaining to evaluate the change in vessel density and vessel coverage by pericytes. Second, we investigated angiogenic target genes whose translation could be impacted by anti-miR-21 therapy. We performed quantitative polymerase chain reactions (qPCRs) on two pancreatic tumor cell lines (BXPC3, PANC-1) and one endothelial cell line, Human Umbilical Vein Endothelial Cells (Huvecs), all transfected with anti-miR-21 for 48 hours. The results of our qPCRs on both Huvecs and Panc-I indicated a decrease in most of the relevant target genes responsible for angiogenesis. Our next step will be to perform a co-culture with Panc-1 and Huvecs to determine how interactions between these cells are modulated in the context of miR-21 therapy.

Cystic Fibrosis Disease Modeling of CFTR Mutation Within Immortalized Human Bronchial Epithelial Cells and IPS Clone Cells

Sang Vo

Principle Investigator: Carla Kim, PhD

Mentor: Hongmei (Lisa) Li, PhD

Boston's Children Hospital

Cystic fibrosis (CF) is a genetic disorder that affects more than 70,000 patients worldwide, with approximately 1,000 new cases of CF diagnosed each year. CF primarily affects the lungs and patients with CF suffer from symptoms such as difficulty in breathing and frequent infections from developing thick mucus within their lungs. CF occurs from the mutation within the cystic fibrosis transmembrane regulator (CFTR) gene, with the ΔF508 mutation commonly affecting 70% of CF patients. Among the 600 CF patients at the Boston's Children Hospital, five patients were identified being long term non-progressors (LTNP) despite being homozygous for the ΔF508 mutation. Following whole exome sequencing, it was discovered that they also have mutations in other ion channels, with four out of the five patients sharing the mutation within the ENac ion channel. To validate this mutation found in the LTNP as a potential gene modifier for CF, two disease models were developed. The first one was an LTNP CF patient derived induced pluripotent stem cell (iPSC) model with a lung specific Nkx2.1-GFP reporter cloned in, while the other model being from an immortalized human bronchial epithelial cell (iHBEC) line. To obtain ciliated cells within lung organoids derived from iHBEC, two different small molecules known to promote ciliated cell growth in mouse lung organoids will be tested. Using PCR analysis, iPSC clones with a heterozygous knock-in for the Nkx2.1-GFP reporter will be identified. These iPSC clones will then be sorted for lung progenitor cells and differentiated into patient-specific lung organoids. The two-independent 3D lung cell-culture models will have their ion channel function tested. By studying these two CFTR disease models through physiologic assays, new methods could be developed to target CF.

Identification of Important Regulatory Regions of an Enhancer Active in the Developing Retina

Kamari Weaver #

Principal Investigator: Connie Cepko, PhD

Mentor: Brian Rabe

Harvard Medical School

The retina is part of the central nervous system located in the back of the eye which receives and processes visual information. Enhancers are short regions of DNA bound by transcription factors that regulate transcription of nearby genes. The enhancer of interest in this experiment was upstream of neurogenin2 (ngn2) which is active in cells giving rise to rod photoreceptors and amacrine interneurons in the retina. In order to identify important regions of this enhancer, the enhancer was truncated and cloned into a cre-expressing construct and used to label cells with a history of enhancer activity. Following this experiment there was history of truncated enhancer activity in new cell types, bipolar interneurons and Muller Glia, while also a loss of labelled amacrine cells. I conclude that the removed portions likely contained both a binding site for a repressor in bipolar interneurons and Muller Glia and a binding site for an activator in amacrines. Moving forward the ends of the truncated enhancer will be added back individually and tested for regained activity and specificity.

Notable Achievements 2017

In the past year a CURE student:

- Attended the Society for the Advancement of Chicanos/Hispanics and Native Americans in Science conference in Long Beach, California.
- Presented their summer research at the following conferences - 2017 New England Science Symposium- Boston, MA; 2016 Annual Biomedical Research Conference for Minority Students, Tampa, Florida; Worcester Polytechnic Institute Next-In Bio research symposium
- Obtained an award for an oral presentation from Annual Biomedical Research Conference for Minority Students in Tampa, Florida
- Became a recipient of American Society for Investigative Pathology (ASIP) 2017 Gotlieb Undergraduate Student in Pathobiology Travel Award to attend the ASIP 2017 Annual Meeting in Chicago, IL
- Furthered their education at one of the following institutions: Harvard College; Harvard Medical School Biological Biomedical Sciences PhD program; Columbia University; Boston College; Boston University, UMass Amherst, Northeastern University – Masters of Biotechnologies and Pharmaceutical Technologies, UMass Dartmouth, UMass Boston – Honors College, Regis College Tufts University, Boston University School of Public Health and the University of Cambridge
- Was awarded an academic scholarship from the Independent University Alumni Association of Lowell
- Secured employment in the Department of Biostatics and Computational Biology at the Dana- Farber Cancer Center
- Received the 2017 Hope Scholarship Recipient from the Biomedical Science Careers Program
- Returned to the Phyllis F. Cantor Center for Research in Nursing and Patient Care Services to conduct nursing research
- Graduated from one of the following colleges or universities – UMass Boston, Georgetown University; SUNY Downstate Medical School; Smith College, Mount Holyoke, Tufts University, Wheelock and Amherst College
- Was selected as a Semifinalist in the 2016 Siemens Competition in Math, Science, and Technology for her summer research project.
- Received a scholarship from the Newton-Needham Regional Chamber
- Obtained a Fulbright Research Scholarship to examine preventative healthcare access in Rural Areas in India.
- Participated in summer internship program at Sanofi Genzyme
- Received a scholarship from the Alray Scholars Program to provide financial support and mentoring at her undergraduate institution

The CURE Program thanks:

A. David Mazzone Awards Program

Biogen Foundation

Dana-Farber/Harvard Cancer Center and University of Massachusetts Boston U54 Comprehensive Cancer Partnership Program

Friends of Dana-Farber Cancer Institute

Massachusetts General Hospital Cancer Center

National Cancer Institute Cancer Center Support Grant 3P30CA006516-52

Merck & Co., Inc. - Neighbor of Choice

Vertex Pharmaceuticals

The research programs of:
Edward J. Benz, Jr. MD
Lowell Schnipper, MD
Marc Vidal, PhD

Dana-Farber/Harvard Cancer Center's Continuing Umbrella of Research Experiences (CURE) wishes to acknowledge and thank the CURE Advisory Committees, principal investigators, mentors, scientific advisors, lecturers, journal club facilitators and supporters for expanding the career horizons of our students.

Many thanks to the following organizations:

Biogen - Community Lab
Harvard Integrated Life Sciences
Hale Reservation
Office of Diversity Inclusion and Community Partnerships, Harvard Medical School
Les Cordées de la Réussite, Université Paris Est Créteil

A special thanks to:

Tahirah Abdullah, PhD
Fiona Aguilar
Ali Ahmed
Chidi Akusobi
Katrina Armstrong, MD
David Aronstein, MSW
Alazar Ayele
Gonzalo Bacigalupe, EdD, MPH
Our Beyond Academia
Career Representatives
Makena Binker-Cosen
Kristin Bittinger, JD
Simona Bor
Candace Burns, MS
Chérie Butts, PhD
Peter Cabeceiras
Tracy Callahan, PhD
Roger Carter, MS
Danielle Coller
Danielle Cook, PhD
Will Curry, MD
Avery Davis
Tiffany Donaldson, PhD
James Eaglesham
Senait Efrem
Emily Erickson
Blair Evans
Aloisia Fernandes, MS
Erica Fletcher, EdM
Omar Gandarilla, MD
Ashley Gartin
Maud Gilles, PhD
James Gould, PhD
Apurva Govande
Winta Haile
Eric Hall, PhD
Laura Hayman, PhD, RN
Tania Hernandez
Claudia Heske, PhD
Ted Hilaire
Rachada Hiranyaket, MSW
Alexander Jeremiah
Kaiah Jno-Finn
Tatyana Johnson
Dennis Jones, PhD
Abby Joseph
Ruth Kiros, MPH
Julia Knox, MPH
Kelly Kohler, PhD
Alden Landry, MD
Christian Lawrence, MS
Stephania Libreros, PhD
Paul Lipton, PhD
Garvey McKenzie
Sara Miller, PhD
Christophe Morin, PhD
Ryan Mudawar, MS
Barry Nelson
Khang Nguyen
Carl Novina, MD
Mary Nunes
Richard Oakley
Oreofe Odejide, MD
Jessica Olive
Elyse Park, PhD
Obed Posada
Christine Power, MS
Jaqueline Presedo
Shoba Ramanadhan, ScD
Hembly Rivas
Laura Roach
Sarah Rozelle, PhD
John Ryan
Brianne Ryan, MBA
Madeline Scammell, DSc
Jeffrey Smith, MS
Roodolphe St. Pierre
Amanda Stupakevich, MA
Felix Umetiti
Meghan Underhill, PhD, RN
Marina Watanabe
Diedra Wrighting, PhD

NCI
Designated Comprehensive Cancer Center

Dana-Farber/Harvard Cancer Center