An Observational Study on Breast Size Metrics for a Seattle Cancer Screening Population
Payton Mark (Payton) Christiano, Senior, Bioengineering
Siying (Lysia) Li, Senior, Informatics: Data Science
Mentor: Larry Pierce, Radiology, UW Medical Center
Mentor: Paul Kinahan, Radiology, UW Medical Center

Current trends in the healthcare industry are focused on providing precision medicine for more individualized patient care. Many researchers are actively designing specialized devices specific to the imaging and care of breasts. However, we are unaware of any comprehensive large-scale studies that analyze population breast sizes that provide design guidelines for such precision medicine machines. In order to fill this knowledge gap, we collected nearly 15,000 mammograms from the Seattle Cancer Care Alliance and built a database of the images and DICOM (Digital Imaging and Communications in Medicine standard) header information. Images which were selected to be analyzed were run through a MATLAB algorithm to extract length and width measurements of each individual breast in the General Electric mammography unit. Around 2,400 images with Cranio-Caudal view, without magnification, and without foreign objects, were run through the MATLAB algorithm. With the measurement results from this algorithm, we studied the statistical distribution of the breast size and shape from this population. The extent of the breasts from the chest wall into the scanner had a mean of 96.19 +/- 29.04 mm , while the width of the breast had a mean of 184.69 +/- 29.00 mm. With the database in place, the next phase of our research was dedicated to analyzing breast density among the population. Women with dense breasts are at higher risk of false negatives in cancer screening and many states require that clinicians notify a woman if she has dense breasts. Nevertheless, there exists the problem of intra- and interobserver variability for judgement of breast density. To address this, some researchers have proposed automated algorithms to quantify breast density. We used our existing database to analyze the variability within these automated algorithms.

Outcomes among Merkel Cell Carcinoma Patients with Chronic Immunosuppression
Maclean M (Maclean) Cook, Senior, Neurobiology
UW Honors Program
Mentor: Paul Nghiem, Dermatology
Mentor: Yolanda Tseng, Radiation Oncology, UW Medical Center

Merkel cell carcinoma (MCC) is a rare, aggressive neuroendocrine skin cancer, which primarily afflicts Caucasian and elderly patients. While MCC patients with intact immune systems have a 20-30% risk of recurrent MCC within 2 years of diagnosis, risk of recurrence or death is higher among immunosuppressed patients. However, it is unknown whether immunosuppression type affects progression-free and disease-specific survival. From a large data repository, we identified and retrospectively evaluated 95 MCC patients that were chronically immunosuppressed at the time of diagnosis and were treated with curative intent between 1988 and 2016. There were five types of chronic immunosuppression represented: chronic lymphocytic leukemia (CLL, 29% of immunosuppressed cases), solid organ transplant (SOT, 23%), autoimmune disorders (AD, 22%), other hematologic malignancies (OHM, 17%), and HIV/AIDS (9%). Progression free survival (PFS) and disease specific survival (DSS) were estimated by the Kaplan-Meier method. As part of curative-intent therapy, the majority of patients were treated with wide local excision (89%) followed by adjuvant radiation (82%). Immunosuppression was associated with poor outcomes among MCC patients: 72% of such MCC patients experienced recurrent disease at a median time of 7.4 months after diagnosis (range, 1-75 months). 45% of recurrences in immunosuppressed patients were distant, while 30% were regional and 25% local/in-transit. 2 yr-PFS and DSS by immunosuppression type was: CLL (PFS 20%/DSS 59%), SOT (0%/32%), AD (14%/52%), OHM (29%/43%), and HIV/AIDS (0%/0%). Immunosuppression is associated with a high risk of recurrence and mortality in MCC patients. While these results are preliminary, they suggest that the type
of chronic immunosuppression may affect outcomes in MCC patients.

**Deriving Dendritic Cells from Acute Myeloid Leukemia Blasts for Immunotherapy**

*Hunter Avery Culp, Senior, Biology (Physiology)*  
*Mentor: Marie Bleakley*  
*Mentor: Melinda Biernacki, Clinical Research Division, Fred Hutchinson Cancer Research Center*

Acute myeloid leukemia (AML) affects people of all ages and, despite aggressive treatment, more than a third of patients relapse, creating the necessity for novel therapies. Immunotherapies have successes in treating other cancers, but have not yet been fully developed for AML. Dendritic cells (DC) are potent antigen-presenting cells that can stimulate cytotoxic T cells and can be derived from leukemic blast cells (AML-DC). AML-DC have the potential to prime and expand leukemia-specific cytotoxic T cells for use in immunotherapies. DC can be generated in the laboratory using a standard protocol in which CD14+ monocytes are exposed to inflammatory cytokines (CD14-DC). However, most AML blast cells are CD14- and an alternative selection process must be used. CD33 is a myeloid marker present on monocytes and on AML. To develop an appropriate control for AML-DC, we hypothesize that CD33 selected cells from normal donors can be used to generate DC (CD33-DC) with similar properties to those from CD14+ monocytes. CD14-DC and CD33-DC from normal donor peripheral blood mononuclear cells showed similar expression of the DC maturation markers CD80 and CD86 (97% vs. 95% and 99% vs. 90% respectively for CD14-DC vs. CD-33) but only 63% of CD33-DC expressed CD83 vs. 87% of CD14-DC, which could indicate that CD33-DC are less capable of stimulating T cells. To compare functionality of CD33-DC, we used CD33-DC and CD14-DC to present known antigens to CD8+ T cells and evaluated expansion of antigen-specific T cells by the DC. Additionally, we compared cytokine production by CD33-DC and CD14-DC. Determining if CD33 selection is capable of producing DC in normal donors gives us the necessary controls to use for comparison to AML-DC. We can use endogenously expressed antigen from AML-DC to find leukemia-specific T cells to be developed for T cell immunotherapies to cure AML.

**Optimization of Chlorotoxin Conjugation to Nanoparticle: Targeting Chemotherapeutics to Glioblastomas**

*Dua Naveed Khan, Senior, Biology (Molecular, Cellular & Developmental)*  
*UW Honors Program*  
*Mentor: Miqin Zhang, Materials Science & Engineering*

Glioblastomas (GBMs) are malignant brain tumors associated with lethal cancers that have a great tendency to rapidly invade healthy brain tissue, leaving patients with short survival times and decreased quality of life. The median survival time for patients treated with the chemotherapeutic, temozolomide (TMZ), and radiation therapy is around 12 months, and many patients develop resistance to TMZ due to high activity of O6-methylguanine-DNA methyltransferase (MGMT), a DNA repairing protein. However, the inclusion of an MGMT inhibitor, such as O6-benzylguanosine (BGS), could work to combat this problem. Currently, the Zhang lab is working on creating nanoparticles (NP) with drug-delivery capabilities, which can cross the blood-brain barrier and deliver chemotherapeutics to targeted glioma cells. This highly drug loaded NP model (IOPH-pBGS) efficiently inhibits MGMT and sensitizes GBM cells to TMZ. However, we wanted to know to what extent a targeting agent would improve this model, and how much efficacy can be increased through optimization of the targeting agent conjugation process. We hypothesized that we can significantly increase drug delivery to tumors by avoiding nonspecific uptake of NPs in healthy cells. My research project was to optimize chlorotoxin (CTX) conjugation to the NPs for use against GBMs. CTX is a small amino-acid peptide sequence that has the ability to target GBM cells. We optimized: the point in the NP synthesis process to add CTX, the length of polyethylene glycol linker to attach CTX to the NP surface, and the reaction ratio of CTX to NP in order to optimize both drug loading and targeting efficacy. In order to evaluate the effectiveness of the various CTX conjugated NPs, SDS-PAGE peptide quantitation assays, cell targeting assays, MGMT quantitation assays and clonogenic cell survival assays were utilized.

**Identification of Mutations Induced by Radiation and HER2 Overexpression in Breast Carcinogenesis**

*Seung Hyuk (Tony) Lee, Senior, Chemistry, Biochemistry*  
*Mentor: Eun Hyun Ahn, Pathology*

Exposure to ionizing radiation is the longest-established environmental cause of human breast cancer in both women and men. Evidence indicates that HER2-positive breast cancers are more likely to spread and are more resistant to anticancer-treatments. The goal of our study is to investigate genetic variations that are introduced during breast carcinogenesis. As a progressive stages of breast carcinogenesis cell model, normal human breast stem cells were sequentially transformed with SV40 large T antigen, x-ray radiation, and HER2 (ERBB2) overexpression to generate non-tumorigenic/immortalized cells, weakly tumorigenic cells, and highly tumorigenic cells, respectively. We sequenced 82 genes that are frequently mutated in breast cancer using next generation sequencing. We identified 26 radiation-specific mutations in exonic or intronic splicing regions that are not present in immortalized cells, but are present only in weakly tumorigenic cells. Among the 26 mutations, a single nucleotide deletion in RECQL gene, which is involved in DNA
repair, is highly mutated (43%). Mutations induced by the overexpression of HER2 were identified by analyzing mutations present in highly tumorigenic cells but not in weakly tumorigenic cells. Two HER2 overexpression-specific mutations PRSS1 p.K225R and RET p.F683Y were identified with the mutation RET p.F683Y present at a high prevalence of 54%. The RECQL deletion induced by radiation and the two nonsynonymous mutations PRSS1 p.K225R and RET p.F683Y induced by HER2 overexpression have not been previously reported by others. Studies are underway to further characterize genetic variations that are induced by SV40-T Ag, radiation, and HER2 overexpression in breast carcinogenesis. The identified mutations that are specific to these oncogenic treatments aid in characterizing molecular subtypes of breast cancers and provide a foundation of identifying potential target mutations that can serve as biomarkers for the sequential stages of breast carcinogenesis.

**In Vivo Screen for Drugs that Can Target Cancer Stem Cells Utilizing Drosophila Germ-Line Stem Cell Model**

Ariene Rivas, Sophomore, Pre-Health Sciences  
Ermyas Asfha (Ermyas) Kahsai, Senior, Biology (Bothell Campus)  
Nahom Yilma (Nahom) Seyoum, Senior, Biology (Bothell Campus)  
Deepkiran Singh, Senior, Seyoum, Senior, Biology (Bothell Campus)  
Mentor: Fillipo Artoni, Biochemistry

Aggressive cancers are resistant to apoptosis, and stem-like cancer cells might be responsible for re-growth of cancerous tumors. This inspires the search for compounds that could aid in the destruction of cancer cells with stem-like properties. Drosophila germ line stem cells under radiative or chemotherapeutic stress are protected by a molecular signal from apoptotic daughter cells via the TIE receptor. Thwarting this protective signal by the introduction of a small molecule may lead to the discovery of compounds that can be effective against stem-like cancer cells. The National Cancer Institute (NCI) diversity set of 1,596 small molecules is screened, in vivo, to find compounds that can induce apoptosis in the germ-line stem cells of Drosophila melanogaster. We tested several compounds from the NCI diversity set on 0-5 day-old fruit flies to get an understanding of the effects of the compounds on stem cells. Multiple compounds, including Campothecin and Maytansinol, have been found that can induce apoptosis in both the male and female germ-line stem cells. Future investigations will uncover the mechanism of action and efficacy against stem-like cancer cells. After determining which compounds work effectively to target stem cells, this knowledge will be applied to understanding the mechanism responsible for resistance to cell death by stem cells and potentially some cancer cells, which could lead to more effective cancer-eradicating drugs.

**Investigating the Role of Proline-Rich Tyrosine Kinase 2 (Pyk2) in Epithelial-Mesenchymal Transition and Tumor Metastasis**

Jiye (Stella) Shin, Senior, Biochemistry  
Mentor: Taran Gujral, Human Biology, Fred Hutchinson Cancer Center

The epithelial-mesenchymal transition (EMT) is a reversible process in which epithelial cells adopt mesenchymal properties by altering their morphology, adhesion, and migratory capacity. During tumor progression, the EMT underlies a process that allows benign tumor cells to infiltrate surrounding tissue and metastasize to distant sites. Our lab has identified a non-canonical Wnt signaling that involves ligands (Wnt 5a/b) and Frizzled2 receptor (Fzd2) in regulating EMT as well as tumor metastasis. Inhibition of Fzd2 signaling suppresses EMT and the progression of metastatic cancer cells both in vitro and in vivo. Recently, we have discovered a non-receptor proline-rich tyrosine kinase 2 (Pyk2) that plays a critical role in Fzd2-mediated cell migration. However, details of Fzd2-mediated activation of Pyk2 and mechanistic understanding of Pyk2 interactions in the pathway still need to be elucidated. We hypothesized that phosphorylation of Fzd2 induces activation of Pyk2, a possible contributor in Fzd2-mediated EMT pathway and tumor metastasis and aimed to uncover 1) the role Pyk2 in Fzd2-mediated EMT induction and cancer metastasis 2) the new mechanistic understanding of how Pyk2 interacts with proteins in the Fzd2 complex. Our preliminary data showed that inhibiting kinase activity of Pyk2 downregulated mesenchymal markers such as vimentin and upregulated epithelial markers such as E-cadherin. Then, the study focused on perturbing Pyk2 expression by RNAi and assessing EMT markers, and cell motility as well as gaining a mechanistic understanding of how Pyk2 interacts with other proteins in the complex. A better understanding of Fzd2-Pyk2 signaling would not only help in connecting critical nodes in Wnt pathway but may open up potential treatment options for metastatic cancers.

**Development of a Renal Cell Carcinoma Kidney-Tumor-on-a-Chip to Mimic Tumor-Induced Angiogenesis**

Connor Andrew (Connor) Tsuchida, Senior, Bioengineering  
Mary Gates Scholar, UW Honors Program, Undergraduate Research Conference Travel Awardee, Washington Research Foundation Fellow  
Mentor: Ying Zheng, Bioengineering  
Mentor: Chris Miller, Medicine

Kidney cancer is the 7th and 10th most common cancer in men and women in the United States respectively. Renal cell carcinoma (RCC), a cancer of the proximal convoluted tubule, accounts for >90% of these kidney cancer incidences.  

Connor Andrew (Connor) Tsuchida, Senior, Bioengineering  
Mary Gates Scholar, UW Honors Program, Undergraduate Research Conference Travel Awardee, Washington Research Foundation Fellow  
Mentor: Ying Zheng, Bioengineering  
Mentor: Chris Miller, Medicine

Kidney cancer is the 7th and 10th most common cancer in men and women in the United States respectively. Renal cell carcinoma (RCC), a cancer of the proximal convoluted tubule, accounts for >90% of these kidney cancer incidences.  

Connor Andrew (Connor) Tsuchida, Senior, Bioengineering  
Mary Gates Scholar, UW Honors Program, Undergraduate Research Conference Travel Awardee, Washington Research Foundation Fellow  
Mentor: Ying Zheng, Bioengineering  
Mentor: Chris Miller, Medicine

Kidney cancer is the 7th and 10th most common cancer in men and women in the United States respectively. Renal cell carcinoma (RCC), a cancer of the proximal convoluted tubule, accounts for >90% of these kidney cancer incidences.
Most cancers, including RCC, stimulate angiogenesis – the sprouting of existing blood vessels. By upregulating the expression of angiogenic factors, such as vascular endothelial growth factor (VEGF), tumors promote vascular sprouting within their microenvironment. This increased vasculature allows for increased oxygen transport as well as an opportunity to metastasize. This tumor-induced angiogenesis is of particular interest to researchers hoping to continue to develop alternative cancer therapeutics to inhibit angiogenesis, starving the tumor of oxygen, or normalize the vasculature, making drug delivery more effective. Common cancer models like 2D cell cultures and animal xenografts have been shown to be poor predictors of drug efficacy in patients. A lack of cellular complexity in 2D cultures along with genetic differences in animal xenografts make them insufficient models of the human pathophysiology seen in vivo. To address this problem, we are developing a 3D, vascularized, flow directed, microphysiological device deemed a “kidney-tumor-on-a-chip”. To engineer a perfusable microvessel, a 120\(\mu\)m collagen channel was lined with human umbilical vein endothelial cells (HUVECs). Primary RCC spheroids that mimic the 3D structure of solid tumors were then introduced into the surrounding collagen, recapitulating the tumor-vessel microenvironment. Initial results have shown no endothelial sprouting in non-RCC control devices, compared to significant endothelial sprouting in devices with RCC spheroids. These results suggest RCC cells may contribute to angiogenic sprouting in our microphysiological device, simulating what is observed in vivo. The ability of this kidney-tumor-on-a-chip to mimic the tumor-vessel microenvironment demonstrates its potential to be a more effective cancer model for drug testing.