The Expression of Adhesion Molecules in CD19-Directed CAR T Neurotoxicity Mouse Model
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Mentor: Juliane Gust, Neurology

Chimeric antigen receptor (CAR) T cell therapy is a highly effective treatment for blood cancers; however, it is correlated with neurotoxicity in about 40% of cases. Neurotoxicity manifests as headaches, delirium, and in more severe cases, seizures, coma, and death. In a mouse model of CD19-directed CAR T cell therapy, we observed that mice developed behavioral changes, as well stalled blood flow in over 10% of brain capillaries. This was caused by circulating leukocytes becoming stuck in the capillaries. My goal for this project was to investigate the underlying mechanism of capillary stalls in the context of CAR T cell therapy. We predicted that capillary stalls are caused by increased expression of adhesion molecules, such as Intercellular Adhesion Molecule-1 (ICAM-1) and Vascular Cell Adhesion Molecule-1 (VCAM-1), by the endothelial cells that comprise the capillary walls. These adhesion molecules grip onto molecules on the surfaces of leukocytes, creating plugs and halting blood flow through the vessels. To test this hypothesis, I used immunohistochemistry to compare the expression of ICAM-1 and VCAM-1 between 4 treatment groups: mock, tumor, CAR T, and tumor+CAR T. Mice from each treatment group were perfused and their brains were collected and sectioned. I incubated mouse brain sections in a primary antibody solution targeting ICAM-1 and VCAM-1 between 4 treatment groups: mock, tumor, CAR T, and tumor+CAR T. Mice from each treatment group were perfused and their brains were collected and sectioned. I incubated mouse brain sections in a primary antibody solution targeting ICAM-1 and VCAM-1, and then in a secondary fluorescently conjugated antibody solution that bound to the previous antibodies, enabling specific labeling of the molecules of interest. I then imaged the sections with a confocal microscope and analyzed them using ImageJ software. I quantified the brightness of fluorescence of ICAM-1 and VCAM-1 to determine the relative expression of these molecules between control mice and those receiving CAR T. The findings from this project have contributed to our overall understanding of the mechanisms of neurotoxicity associated with CAR T cell therapy.

Identification of MHC-2+ Cells in the Niche of Dormant Disseminated Tumor Cells
Marissa Karina Guerrero, Junior, Psychology
Mentor: Cyrus Ghajar, Translational Research Program, Fred Hutchinson Cancer Research Center
Mentor: Erica Goddard

Breast cancer is one of the most common types of cancer in women, resulting in ~42,000 deaths annually in the United States. Tumor cells can travel to distant tissues and form new tumors, a process known as metastasis, which ultimately kills the patient. These disseminated tumor cells (DTCs) that stray from the original tumor may undergo a period of dormancy prior to awakening and growing into lethal metastases. Dormancy is when a DTC is in a non-dividing (i.e., quiescent) state, but remains receptive to microenvironmental cues that trigger proliferation. The relationship between DTCs and their surrounding immune microenvironments is not well understood, thus we opted to explore whether dormancy of DTCs is impacted by the immune milieu. We used the mouse lung as an experimental model. Following intravenous injection of D2.0R mammary tumor cells, we stained for MHC-2 to identify myeloid and B cell populations in the lung. Surprisingly, we found that MHC-2+ immune cells border dormant DTCs. We performed a control analysis protocol by placing random dots (representing random cells) in the stained tissue images and found that MHC-2+ cells are not in immediate proximity to our random ‘cells’, suggesting that the association between MHC-2+ cells and DTCs is likely not arbitrary. Our next step in this investigation is to identify precisely what these MHC-2+ cells are, looking specifically at markers for myeloid and B cell subsets including F4/80, CD11c, and CD19. This study will provide us with a better understanding of the relationships between dormant DTCs and immune cells in their immediate lung microenvironment. Our long-term goal is to use what we learn from these studies to aid in the development of immunotherapies to target DTCs and prevent metastasis.
Cytotoxicity of BCL-xL and MCL-1 Inhibitors for Treatment of Non-Hodgkin’s Lymphoma

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Mentor: Shyrl O’Steen, Clinical Research, Fred Hut
Mentor: Damian Green, Medicine, Fred Hutchinson Cancer Research Center

Non-Hodgkin’s Lymphoma (NHL) is the most common malignancy of the blood and bone marrow, responsible for about 4% of all cancers. The anti-apoptotic MCL-1 and BCL-xL proteins have been linked to the development of various types of NHL. Radiation is an effective treatment, as it is known that lymphomas are generally highly sensitive to radiation, but toxicity can limit the dose. Our goal is to use different treatments in varying combinations to find the best synergy where the effect of the treatments surpasses the expected additive response. We first tested in vitro response of NHL cell lines to the BCL-xL inhibitor A1155463 and the MCL-1 inhibitor S63845. We then evaluated combinations between the two inhibitor drugs with radiation treatments. To date we have tested cytotoxicity of these treatment combinations in six NHL cell lines. All lines tested with the combination of the MCL-1 and BCL-xL inhibitors demonstrated an increase in cell death attributable to a synergistic effect between the two drugs. Furthermore, when treated with radiation prior to the two drug treatments, three out of the six lines demonstrated a synergistic response to the triple treatment combination. The other three lines displayed an additive response to the triple combination. Moving forward we aim to test a total of 14 NHL cell lines, and given the positive results to date, will also expand the work into mouse models. These largely synergist results show promise because they suggest that this triple combination treatment could reduce treatment side effects while also improving the curative response for NHL patients.

Overcoming Cell Therapy Barriers in the Suppressive Tumor Microenvironment With Engineered Switch Receptors

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Mentor: Shannon Oda, Pediatrics, Hematology/Oncology
Mentor: Edison Chiu

Adaptive cell immunotherapy (ACT) with engineered T cells has shown impressive efficacy against some cancers, particularly CD19+ leukemias. However, ACT efficacy in solid tumors can be limited by restrictive tumor microenvironments (TMEs), with increased inhibitory signals, reduced T cell infiltration/accumulation, and inadequate metabolic substrates. We engineer T cells with novel switch receptors that combine an inhibitory ectodomain with a costimulatory signaling endodomain, to “replace a break with an accelerator”. FasL is a death receptor ligand that is overexpressed in the majority of human TMEs and can protect tumor cells from immunity by giving T cells inhibitory signals upon binding. We engineered a Fas-4-1BB switch receptor to convert the Fas death signal to a costimulatory 4-1BB signal and demonstrated enhanced Fas switch-receptor-T cell in vivo persistence and therapeutic efficacy in a murine pancreatic cancer model (KPC). We developed new switch receptor candidates by combining CD200R, an inhibitory signaling receptor, ectodomain with various costimulatory endodomains. Our aim is to screen for CD200R switch receptors that exhibit the best expression on T cells, best ability in tumor-lysis and proliferation with the least exhaustion in TMEs, and test the best candidates in KPC models. We are able to transduce mouse T cells to express our CD200R constructs, which is verified by flow cytometry, and ready to start in vitro killing assay to assess their ability in tumor-lysis. This T cell engineering strategy may help overcome the restrictive TMEs, catalyzing an endogenous immune response, and greatly improve T cell anticancer efficacy.

[Unable to Present] Refining Oligonucleotide-Directed Biotinylation (ODB), a New Technology for Interrogating Architectural RNAs

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Mary Gates Scholar
Mentor: David Shechner, Pharmacology

Decades of research have demonstrated that RNA molecules can serve as architectural scaffolds, templating the assembly of subcellular compartments in all kingdoms of life. In mammals, architectural RNAs scaffold an array of subnuclear structures that are essential to cellular function including metabolism, DNA repair, and epigenetic programming. Many of these architectural RNAs are causally dysregulated in diseases, including cancer and neurodegenerative disorders. However, the molecular mechanisms of these architectural RNAs remain poorly understood, partially because technologies for identifying the molecules (proteins, DNAs, other RNAs), with which RNAs interact are lacking. To address this challenge, the Shechner Lab has developed a technology termed Oligonucleotide-Directed Biotinylation (ODB), a universal method for elucidating RNA subcellular interactions. ODB applies a powerful method called proximity-biotinylation to individual RNAs. In proximity-biotinylation, a promiscuous biotinylating enzyme (e.g. Horseradish Peroxidase, HRP) is targeted to a subcellular compartment of interest. This enzyme then tags nearby (10 nm) molecules with biotin, enabling their straightforward isolation and analysis. ODB advances this technology by using RNA-in situ Hybridization (RNA-ISH–FISH) methods to precisely deploy HRP to individual RNAs. Ongoing work, using a series of model RNA targets, has demonstrated that OBD can reveal the proteins, RNAs, and genomic loci near a target RNA at exceptional depth and precision. My goal is to generalize this ODB protocol, developing methods that can be applied to any RNA
target. To examine how RNA abundance influences ODB experimental design, I use pulse-chase, RNA decay experiments to manipulate the expression of the architectural RNA NEAT1, and examine how to adjust parameters of the ODB protocol to compensate for this altered expression. Likewise, I am using imaging-based assays to investigate how ODB’s biotinylation radius varies with labeling conditions, increasing the range of ODB’s target radii. Collectively, this establishes general guidelines for adapting ODB to novel RNA targets.

A Machine Learning Approach in Predicting the Behaviors of Cancer Signaling Pathways

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Mary Gates Scholar
Mentor: Herbert Sauro, Bioengineering
Mentor: Lucian Smith, Bioengineering

Cancer is a disease that is characterized by uncontrolled cell growth and proliferation. As these cells spread throughout the body they can invade and damage major organs resulting in death. It has been shown that most cancers arise from genetic changes in the signaling pathways that control the cell growth and proliferation. One major challenge cancer researchers face is to understand the behavior of these pathways. As an example, the behavior of the Endothelial Growth Factor receptor (EGFR) pathway, a commonly mutated pathway in cancer, has a broad range of behaviors. Under different conditions, the pathway can exhibit bistability, amplification and even oscillations in protein levels. Predicting such behaviors can significantly aid our understanding of how cancer arises as well as provide us with insights to therapeutically treat patients. The goal of this project was to develop a high accuracy machine learning model that is capable of predicting the behaviors of signaling pathways. Over the first quarter, I generated over 3000 artificial signaling networks utilizing existing software written by the lab. For each of these networks I then extracted the network architecture, the connections and relationships between species in the pathways, into matrices such as the stoichiometry matrix and scaled Jacobian matrix. These matrices were then treated as pseudo-images and used to train a convolutional neural network to predict oscillatory behavior. We expect this model to have greater than 70 percent accuracy given preliminary models trained on similar smaller datasets. The development of this high accuracy model provides cancer researchers with valuable insights into the behavior of these signaling pathways and how they can best therapeutically treat patients.