2022-2023 St. Jude Summer Plus Projects

St. Jude Faculty Member	Department
Drs. M. Madan Babu & Manbir Sandhu	Structural Biology
Dr. Puneet Bagga	Diagnostic Imaging
Dr. Chi-Lun Chang	Cell and Molecular Biology
Dr. Adam Durbin	Molecular Oncology
Dr. Yongqiang Feng	Immunology
Dr. Marcus Fischer	Chemical Biology & Therapeutics; Structural Biology
Dr. Andrew Heitzer	Psychology
Dr. Lisa Jacola	Psychology
Dr. Jeffery Klco	Pathology
Dr. Chunliang Li	Tumor Cell Biology
Dr. Dirk Loeffler	Hematology
Dr. Heather Mefford	Cell and Molecular Biology
Dr. Charles Mullighan	Pathology
Dr. Stacey Ogden	Cell and Molecular Biology
Dr. Jamy Peng	Developmental Neurobiology
Dr. Jason Rosch	Infectious Diseases
Dr. Lindsay Schwarz	Developmental Neurobiology
Dr. Ranganatha Sitaram	Diagnostic Imaging
Dr. Victoria Willard	Psychology
Dr. Gang Wu	Applied Bioinformatics
Dr. Jun Yang	Pharmacy and Pharmaceutical Sciences

Mechanism aware polygenic risk scores for complex human diseases

Dr M. Madan Babu and Dr Manbir Sandhu
Center of Excellence for Data Driven Discovery
Department of Structural Biology, St Jude Children's Research Hospital

Since the first sequencing of the human genome, genome-wide association studies (GWAS) and data science techniques have been instrumental in identifying how sequence variants in our genomes can cause various diseases in the human population. These tools have identified thousands of genetic regions that are implicated in hundreds of diseases, but there is a strong need for the development of tools that can take us to the next level; we must identify the causal genes and biological mechanisms that lead from genomic variants to disease.

As part of the Center of Excellence for Data Driven Discovery at St. Jude, we are developing novel multi-omics data analysis techniques to integrate signatures from the genomes, transcriptomes, and proteomes of healthy and diseased individuals to better resolve our picture of human health. Toward this, we are developing the use of polygenic risk scores as a method for predicting the gene level effects of disease-associated variants on biological mechanisms, within and across tissues, that are causal in the disease. We term this a "mechanism-aware" polygenic risk score.

The ideal candidate should be an inquisitive problem solver who will thrive in a world class research environment with diverse expertise in biology and data science. The Babu Group strives to build and maintain a culture of respect, diversity, and inclusion and we strongly feel these values directly contribute to the dynamic and intellectually stimulating scientific environment we can provide.

During the Rhodes Summer Plus Program, the mentee will be mentored and trained directly by Dr. Sandhu in:

- Analyzing multi-omics datasets using advanced data science techniques
- Collecting and organizing individual and population level human genomics data from cohort studies
- Using common genetics analysis software tools (e.g. PLINK)
- Building statistical models of gene-trait-disease interactions
- Predicting susceptibility of individuals to specific diseases based on mechanism-aware risk scores

Interaction with other members of the group and other experts within St. Jude who can provide relevant expertise will also be encouraged.

Skills required:

- Experience in scripting languages (Python, R, bash)
- Familiarity with statistical concepts (e.g., probability theory, Bayes' theorem)
- Familiarity with genetics, cellular biology, and molecular biology
- Experience with multivariate and univariate modeling (ideally)
- Excellent written and verbal communication skills

Metabolic Imaging Research Lab – Puneet Bagga, PhD

Metabolic reprogramming is a hallmark of cancer. The reprogrammed pathways contribute to cancer progression by excessive cell proliferation, cell survival and immune evasion. The importance of studying cancer metabolism is emphasized by the fact that non-invasive imaging tools and therapeutic strategies in cancer exploit the altered metabolic states in tumors. The metabolic imaging lab uses metabolomics, metabolic flux analysis, cell biology and patient derived xenograft models of cancer to study how tumor cells metabolize small molecules including glucose, acetate, and glutamine, build macromolecules and proliferate, and maintain cell survival and cellular energetics in severe microenvironment. We develop clinically translatable metabolic imaging tools using multiparametric MRI, proton magnetic resonance spectroscopy (^{1}H MRS) and molecular imaging to identify predictive biomarkers correlating with altered metabolism. Additionally, we perform non-radioactive isotope tracer analysis *in vitro* and *in vivo* to identify key pathways as potential drug targets.

Given the poor outcomes from diffuse intrinsic pontine gliomas (DIPGs) and the potential that metabolic reprogramming may identify new therapeutic targets, a thorough examination of metabolism in DIPGs is warranted. The activation of the PI3K/AKT/mTOR pathway contributes to elevated glutaminolysis, *de novo* fatty acid synthesis and pyruvate entry into the tricarboxylic acid (TCA) cycle. However, the diverse cellular and metabolic consequences of deregulated PI3K signaling in DIPGs are still elusive. We perform ^{13}C tracer studies in patient derived cell lines to measure the flux rates in the key metabolic pathways. Briefly, ^{13}C-labeled glucose is used for measuring flux in downstream glycolysis and TCA cycle, glutamine provides information about anaplerotic pathways, and acetate is used to measure fatty acid synthesis rates. We then combine *in vitro* flux analysis with *in vivo* multiparametric MRI/MRS data in PDX DIPG models to identify and target the metabolic alterations caused by PI3K/mTOR pathway inhibition. This can be used for assessing targeted therapy response and to develop reliable and translatable non-invasive metabolomic biomarkers of cancers with the potential to guide novel therapeutic interventions, including combinatorial therapies.

References:

1. Chung C *et al*., Integrated Metabolic and Epigenomic Reprograming by H3K27M Mutations in Diffuse Intrinsic Pontine Gliomas, Cancer Cell, 2020
2. Rich L *et al*., ^{1}H magnetic resonance spectroscopy of ^{2}H-to-^{1}H exchange quantifies the dynamics of cellular metabolism in vivo, Nature Biomedical Engineering, 2020
3. Tam L *et al.*, MRI-based radiomics for prognosis of pediatric diffuse intrinsic pontine glioma: an international study, Neuro-oncology Advances, 2021

Lab of Chi-Lun Chang

Research Summary:

Nutrients and metabolites need to be distributed carefully between organelles within cells to integrate cellular function and meet metabolic demands in a human body. Our laboratory investigates the formation, organization, regulation, and function of a complex inter-organelle logistic network. We address these questions via cutting edge light and electron microscopy tools in conjunction with a multidisciplinary approach, including molecular and cell biology, biochemistry, *in vitro* reconstitution, real-time metabolic analyses, and genome-editing technology. Our goal is to gain mechanistic insights into this logistic network in normal cells so we can translate this knowledge into the understanding of pathogenesis of diseases.

We are looking for talented scientists to join us. Prerequisites are creativity, passion, dedication, competitiveness, and the ability to work collaboratively in a small group. Candidates with experience in basic cell and molecular biology, biochemistry, and imaging technologies are preferred.

Understanding the formation and regulation of lipid droplet-organelle contact sites

Fatty acids are indispensable materials for lipid synthesis and energy production. Lipid droplets play important roles in fatty acid metabolism via functional alliances with many other organelles at contact sites. However, the molecular basis of lipid droplet-organelle contact sites formation and regulation are largely unknown. This is due, in part, to the lack of tools that can visualize such dynamic nano-architecture, as well as the scarcity of known tethering components. Our team plans to engineer synthetic tools that selectively label lipid droplet-organelle contact sites with minimal perturbation to cells. These tools will enable us to quantitatively describe the dynamics of contact sites in conditions of energy surplus or starvation. We also aim to identify and characterize tethering components by mapping the proteome at these sites using proximity labeling enzymes and by CRISPR-based knockout screens.

Deciphering how the tubulin code collectively regulates organelle distribution involved in energy homeostasis

It is yet unknown how cells coordinate the proximity of organelles to form contact sites in the first place. Evidence suggests that tubulin codes, generated by posttranslational modifications, create identifying landmarks. We hypothesize that the tubulin code dictates the location and duration of lipid droplet-organelle contact sites to regulate trafficking energy homeostasis. We are addressing this question from the perspectives of code-writing enzymes and code-recognizing effectors. We are applying gene editing technology to modulate coding/recognition machinery and analyze distribution, architecture, and function of these contact sites. Aberrant regulation of the tubulin code is associated with neurodegenerative diseases and defective energy homeostasis. Our work stands to provide a new perspective on the pathogenesis of neuron cell dysfunction as it relates to energy homeostasis at lipid droplet-organelle contact sites.

Investigating the formation and function of early secretory compartments

A conserved secretory pathway is responsible for trafficking membrane and secretory proteins made in the endoplasmic reticulum (ER). The first step in this pathway is to sort and concentrate protein cargos into ER exit sites (ERESs). Our previous work revealed a dynamic, complex

tubule network of early secretory compartments in intact mammalian cells and provided an updated conceptual framework for understanding how the secretory pathway functions and adapts to deliver diverse cargo types to the Golgi. We are now interested in understanding how these membrane organizations are formed. Our lab is developing synthetic degradation systems and *in vitro* reconstitution assays to identify critical mediators and recapitulate ERES formation. Ultimately, we aim to understand how this early secretory pathway functions and adapts to accommodate metabolic demands.

The Durbin Lab in the Division of Molecular Oncology at St. Jude Children's Research studies the fundamental mechanisms that drive children's cancer. While pediatric cancer outcomes have improved in some diseases, children with solid tumors continue to do poorly with little improvements over the past decades. **In many of these diseases, no targeted therapies are available.** Our laboratory uses a variety of cutting-edge genome-scale technologies to identify and target the fundamental mechanisms by which high risk pediatric solid tumors grow and metastasize. Our lab bridges chemical biology, faithful animal modeling of human cancer and genome editing to identify and develop new methods to target key cancer drivers. Our approach is rooted in a single, common goal: Identify targets, so we can identify new compounds that we can use to improve cancer outcomes and reduce toxicity.

We have identified several key cancer drivers in high risk pediatric solid tumors in which there are limited options for therapy, such as neuroblastoma and rhabdomyosarcoma. We focus on two main areas:

1. Chemical Epigenetics: We have developed strategies targeting novel epigenetic regulators that control the transcriptome of neuroblastoma (*Molecular Cell* 2020; *Science Advances* 2021; *Cancer Discovery* 2021). We are studying these and other epigenetic regulators using our new targeted compounds, both alone and in combination, for their potent and wide-ranging effects on tumor cell identity and fate.

2. Transcription Factor complexes and control of transcription: We have identified a group of transcription factors that function to establish the malignant identity of neuroblastoma (*Nature* 2015; *Nature Genetics* 2018; *Nature Communications* 2019). We dissect these transcription factor complexes using a variety of technologies, including mass spectrometry-based proteomics, chemical biology, advanced genome editing and imaging-based approaches, to identify key targets for therapeutic inhibition.

Students working in the Durbin laboratory will learn and use a variety of cell culture and molecular biology techniques to work on either of these projects. There are subprojects available in either area 1 or 2, working directly under the supervision of a post-doctoral fellow. In all cases, students will be expected to read the primary literature and develop skills in a mentored environment in cell culture, molecular cloning, compound treatment, western blotting, immunofluorescence, RT-qPCR, mouse modeling of cancer and more advanced technologies including ChIP-seq, CUT&RUN sequencing, RNAseq and proteomics technologies, depending on the demands of the project.

Yongqiang Feng, PhD
Assistant Member, Immunology Department
St. Jude Children's Research Hospital

Subtypes of T lymphocytes play essential, distinct roles in protecting against infection, cancer, or autoimmune diseases. My laboratory aims to identify novel factors and mechanisms controlling T cell differentiation and function. Over the past few years we have developed innovative genetic and biochemical tools (unpublished) to achieve this goal. Students will be guided by the PI and senior postdoctoral fellows to explore these cutting-edge tools and to investigate the new factors recently discovered in the lab under the contexts of T cell anti-autoimmune and anti-tumor activities.

Subtypes of T lymphocytes play essential, distinct roles in protecting against infection, cancer, or autoimmune diseases. We are particularly interested in the balance between regulatory T cells and effector T cells. The former suppress and the latter promote immune response. Maintenance of this balance is essential for immune homeostasis and response to pathogens and tumor. We aim to identify novel factors and mechanisms controlling the differentiation and function of these two major classes of T cell types. Over the past few years we have developed innovative genetic and biochemical tools (unpublished) to achieve this goal. These create many opportunities for students to learn and investigate how T cells are regulated to tolerate or respond to immune challenges. Students will be guided by the PI and senior postdoctoral fellows to use our new genetic tools in combination with a spectrum of cutting edge technologies, including flow cytometry, epigenetic methods, single cell RNA sequencing, and bioinformatics to dissect T cell behaviors in animal models of autoimmune diseases and cancer. They may also choose to integrate CRISPR genome editing with tumor models to investigate how the new protein factors we recently discovered in the lab control the anti-tumor or anti-autoimmune activities of T cells. Please contact us for more details regarding specific projects.

Revealing invisible differences in near identical proteins

PI: Marcus Fischer, PhD, Department of Chemical Biology & Therapeutics and Structural Biology
https://www.stjude.org/research/labs/fischer-lab.html

Protein isoforms are sets of highly similar proteins. They often share very high sequence similarity, are structurally indistinguishable and conduct similar function. Their near identical nature makes it challenging to find small molecules that target individual isoforms specifically. The motivation for this project is threefold: Isoform-selective small molecules would help us better understand subtle differences in their biological role, they would help us target disease-driving isoforms, and they would reduce off-target side effects of current promiscuous binders.

To achieve this we use a spin on common X-ray crystallography that provides key information about structural dynamics at the molecular level. While 95% of all structures in the Protein Data Bank are collected at cryogenic temperatures (100K), we will solve structures at physiologically relevant temperatures. We have shown that this can unmask biologically meaningful conformations and reveal minor differences that can help us distinguish isoforms. The goal of the project is to leverage these insights towards finding isoform-selective small molecules to probe biology and treat disease.

The Rhodes Summer Plus fellow involved with this project will learn state-of-the-art molecular biology and structural biology techniques along the gene-to-protein-to-structure pipeline that are relevant for academic research and industry. No prerequisite skills required, but prior hands-on experience in molecular biology techniques and programming skills for data analysis (Python, R, Matlab) would send you off to a flying start.

References:

• Bradford et al. (2021). Temperature artifacts in protein structures bias ligand-binding predictions. *Chem Sci 12,, 11275-11293.*
• Fischer (2021). Macromolecular room temperature crystallography. *Q Rev Biophys 54, e1.*
• Darby et al. (2019). Water Networks Can Determine the Affinity of Ligand Binding to Proteins. *JACS* 141, 15818-26.

Andrew Heitzer, PhD
Pediatric Neuropsychologist
Instructor
St. Jude Children's Research Hospital

Dr. Heitzer is a neuropsychologist trained to examine brain-behavior relationships in various neurological conditions. His research focuses on neurocognitive functioning in sickle cell disease, an inherited red blood cell disorder with multisystemic effects. Patients with sickle cell disease are at increased risk for numerous brain complications, including stroke and silent infarctions. He has several ongoing projects exploring risk and resiliency factors associated with neurocognitive outcomes in sickle cell disease utilizing biological (e.g. lab values, genetic modifiers) and environmental (e.g. socioeconomic status) predictors. Additional projects examine school readiness and the role of neurocognitive functioning in the transition from pediatric to adult care in sickle cell disease. Students will assist with literature review, data collection, and manuscript/presentation preparation. The student will attend weekly psychology, neuropsychology, and hematology didactics. Opportunities to learn and administer neuropsychological measures are available.

Completion of introductory courses in psychology, neuroscience, research methods, and statistics is preferred. Candidates with prior research experience are desired. The research experience is likely best suited for an individual interested in pursuing graduate school in clinical psychology or medical school.

Lisa Jacola, PhD, ABPP-CN
Pediatric Neuropsychologist
Assistant Faculty Member
Department of Psychology

Jacola Lab – Neurocognitive outcomes and interventions in childhood cancer survivors

Dr. Jacola is a board-certified clinical neuropsychologist and clinician scientist faculty member, with effort devoted to clinical investigation, patient care, and training/supervision. The overarching goal of her research program is to improve neurocognitive and quality of life outcomes in children diagnosed with catastrophic disease. Specific projects are focused on characterizing neurocognitive outcomes, neurocognitive risk factors, and underlying mechanisms in order to inform therapy modifications, guidelines and models for care, and interventions to mitigate deficits. Dr. Jacola has a particular interest in neurodevelopmentally vulnerable populations (i.e., children diagnosed at young ages and children with preexisting neurodevelopmental conditions). Projects focus on populations including survivors of childhood leukemia, survivors of childhood leukemia with Down syndrome, and young children diagnosed with cancer. Ongoing and recently completed projects include: characterizing neurodevelopmental outcomes in very young children diagnosed with cancer, computerized cognitive training during during cancer treatment to prevent cognitive late effects, investigating the impact of early intervention services and socioeconomic status on neurocognitive outcomes, and developing and evaluating aspects of neurocognitive risk screening models. Dr. Jacola has several ongoing neurocognitive studies in survivors of childhood leukemia with Down syndrome, a population that has previously been excluded from neurocognitive studies in childhood cancer.

The student working in Dr. Jacola's lab would assist with literature reviews, study design, data collection and analysis, and dissemination of findings. Mentorship would take place through lab meetings (generally 2/month), supplemented with weekly, one-on-one meetings with study team members to provide an opportunity for one-on-one discussion of learning outcomes. Educational components include Psychology Rounds, Pediatric Neuropsychology Didactics, Jacola Lab meetings.

It would be helpful if students have completed coursework in psychology, neuroscience and statistics. Prior research experience is desired but not required.

Modeling and targeting of pediatric Acute Myeloid Leukemia (AML).

Research in the Klco lab focuses on defining the molecular and biological causes of myeloid malignancies in children. We use a series of genomic approaches (whole genome, whole exome, RNA-seq, single cell genomics) to identify the spectrum of somatic and germline mutations that define these different pediatric malignancies. We further utilize a series of in vitro and in vivo approaches to functionally understand the underlying mechanisms. This includes establishing different genetically engineered mouse models and patient-derived xenograft (PDX) approaches coupled with CRISPR-Cas9 for genome engineering and lentiviral expression models.

Children with AML continue to have a relatively poor overall survival and high relapse rate. This is in part due to the poor understanding of the genomic alterations that initiate leukemia in children and the lack of targeted and personalized therapies. We recently identified a spectrum of genomic alterations in 136 relapsed pediatric AML samples. We are currently working on modeling these alterations in vitro and in vivo. Toward this, the student will work within a team to employ various transcriptomic, proteomic, and cellular assays to identify the pathways involved in these alterations and promote leukemogenesis. Additionally, the project will benefit from the existing collaborations with St. Jude's Chemical Biology & Therapeutics department to identify targeted therapies for these models. Along with the supervision by the lab principal investigator Dr Klco, the student will receive direct training and mentoring from Dr. Sherif Abdelhamed, a senior scientist in our lab with expertise in hematopoietic malignancies. The student will undergo a comprehensive training in cancer biology and hematopoiesis and will acquire technical skills in -but not limited- to colony forming assays, qPCR, western blot, mouse models, flow cytometer, viability assays and high-resolution microscopic imaging. In addition to the active engagement in internal activities provided by SJCRH, the student will benefit from attending the weekly lab meeting, where other members present original research for open discussion and troubleshooting issues.

Functional interrogation of downstream targets of oncogenic *HOXA9* in MLL-rearranged leukemia

PI: Chunliang Li, Ph.D., Assistant Member, Department of Tumor Cell Biology

Research Summary

Aberrant expression of development-associated HOX genes, particularly HOXA9, is a hallmark of most aggressive acute leukemias. These include a large majority of human acute myeloid leukemia (AML) and subtypes of acute lymphoblastic leukemia (ALL), such as refractory MLL-rearranged (MLL-r) ALL and $NPM1c^{+}$ AMLs. Overexpression of HOXA9 predicts not only poor patient survival but also plays a critical role in leukemia development and maintenance. HOXA9 is a homeodomain-containing transcription factor that has been shown to bind genomic regions directly. Oncogenic translocation such as NUP98-HOXA9 retained the DNA-binding domain of HOXA9, again suggesting DNA binding affinity to specific target genes is essential for HOXA9's function in leukemogenesis and tumor maintenance. A growing body of evidence indicates that HOXA9 dysregulation is both sufficient and necessary for leukemic transformation. However, HOXA9 protein itself is a poor therapeutic target as it lacks targetable binding domains. Despite the functional significance of HOXA9 in acute leukemia, the downstream target genes of HOXA9 have not been systematically characterized across leukemias of different genetic subtypes. We have recently established a unique cellular model as MLL-r ALL leukemia cell line expressing the HOXA9-miniAID fusion protein, allowing the acute depletion of HOXA9 protein in hours. We have characterized about 1,800 genomic regions bound by HOXA9 with an isogenic knockout control upon auxin-induced protein degradation by ChIP-seq. In addition, we have conducted a survival-based noncoding CRISPR screen to identify the functional binding sites of HOXA9 and connect the cis-regulatory regulation mechanism to their associated target genes and pathways. We hypothesized that identifying HOXA9-bound genomic loci and their functional target genes is instrumental for understanding HOXA9's regulation mechanism and therapeutic innovation of HOXA9-driven cancers. This project integrates multiple layers of datasets and functional validation to prioritize the novel candidates, allowing the functional interrogation of HOXA9's downstream regulation mechanism in MLL-r leukemia.

This project is designed to prioritize and validate the screen candidates. It will consist of conducting a comprehensive base editor mediated CRISPR editing of *Cis*-regulatory elements (genome-editing), epigenetic characterization of TF regulation (CUT&RUN, ChIP-seq, and ATAC-seq), and leukemia cell biology (*in vitro* leukemia culture, proliferation, and apoptosis) to dissect the molecular regulation network driven by HOXA9 in leukemia. The student will be under one-to-one direct supervision by the PI with technical assistance from other laboratory members. We anticipate these studies will increase our understanding of the mechanisms driving HOXA9-driven leukemia and identify potential therapeutic targets. Welcome to join us!

Loeffler lab

Molecular mechanisms of hematopoietic stem cell self-renewal and differentiation during aging and disease

The balanced production of hematopoietic cells throughout the life of an organism relies on a small number of hematopoietic stem cells (HSCs). While transplantations of HSCs are the only cure for many blood disorders, its application is limited by low HSC numbers and loss of stem cell potential over time. Recent evidence has shown that loss of HSC potential occurs gradually during aging even in healthy individuals and correlates with the number of times a stem cell has divided. The molecular mechanisms behind the loss of stem cell function remain however poorly understood. Understanding how HSC self-renewal and differentiation are regulated and these molecular mechanisms change during aging, inflammation, and disease is thus of great scientific and clinical importance and the focus of my research.

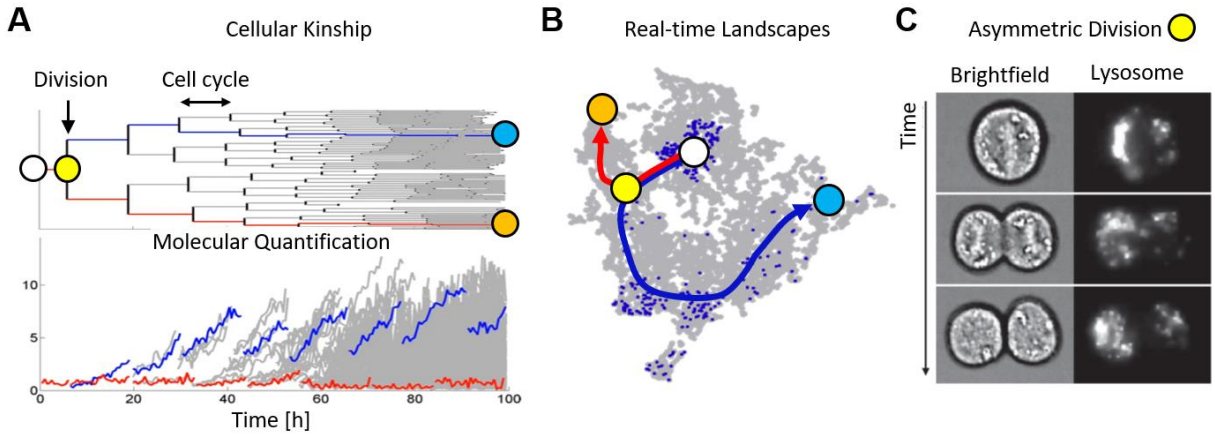

Figure 1: Continuous long-term single-cell bioimaging and quantification. ***a,*** *Example of single-cell genealogy (top) and quantification of marker expression in HSCs (bottom). The analysis of cellular dynamics over time provides an improved understanding of cell fate decision processes.* ***b,*** *Real-time differentiation landscape of HSC differentiation including observed single-cell trajectories.* ***c,*** *Hematopoietic stem cell division with an asymmetric inheritance of Lysosomes (Figure adapted from Loeffler et al. Nature, 2019, Loeffler et al. Blood 2021 and Hoppe, Schwarzfischer & Loeffler et al., Nature 2016).*

Insights into HSC biology are currently derived from population and/or single-cell analysis at discrete time points. However, because rare subpopulations and/or important cellular dynamics are lost, this data can almost always be interpreted in multiple ways and lead to conflicting interpretations. The caveats of snapshot assays are most prominent in highly heterogeneous populations, where subtle, but relevant changes are masked in population averages. It has thus been difficult to decipher the molecular mechanisms and changes regulating HSC activation, aging, and the very first steps of disease initiation. Many aspects of normal and mutant HSC biology thus remain obscure. An improved understanding of these mechanisms requires novel approaches that can quantify individual stem cells over time. My group develops novel hard- and software tools to quantify HSC and Cancer Stem Cell behaviors continuously over many days and cell generations. Using continuous quantitative single-cell analysis, cell behaviors, motility, adhesion, metabolism, differentiation, marker expression, and cell cycle length can be quantified with unprecedented accuracy and single-cell resolution (Figure 1). Using novel bioinformatics tools and machine learning this data can overcome the caveats of snapshot approaches and provide novel insights by observing molecular events as they happen and how they unfold over time. This approach thus can shed new light on the dynamics of molecular processes and provides novel insights about what mechanisms and machinery regulate blood stem cell self-renewal and differentiation in health and disease.

Prerequisite: Highly motivation & ambitious, experience in mol. biology & tissue culture.

Techniques offered: Flow cytometry, time-lapse microscopy, single-cell quantification & analysis

Mefford Lab
Center for Pediatric Neurological Disease Research
St. Jude Children's Research Hospital

The Mefford laboratory is dedicated to identifying and understanding genetic, genomic, and epigenetic causes of pediatric neurological disorders, with a focus on childhood-onset epilepsy disorders called developmental and epileptic encephalopathies (DEE). These are severe conditions characterized by developmental delays and seizures that are difficult to treat. More precise and effective treatments are desperately needed to improve quality of life for individuals with DEE. We believe that identifying the genetic causes helps us understand the pathways that are affected and highlights potential targets for new, gene-targeting therapies.

The lab uses cutting edge technologies including whole genome sequencing and genome-wide methylation arrays to identify disease-causing mutations. We then use genome-engineered cells and cells from patients to study the effects of mutations on gene expression, signaling pathways, and other relevant cellular processes. The cell models we develop, including stem cells and brain organoids, can be used to test potential therapies for specific genetic epilepsies. Project opportunities for a Summer Plus student range from gene and mutation discovery to functional analysis in a cellular model of genetic epilepsy. Students will be paired with a senior lab member, so previous lab experience is not required. Upon completion of their studies, the student will have learned a set of cell biological and genetic techniques commonly used in biomedical research.

Lab of Dr. Charles Mullighan

NUT Midline Carcinoma Family Member 1 Rearrangements in Acute Lymphoblastic Leukemia

Description

This is an exciting career opportunity to join the laboratory of Dr. Charles G. Mullighan, at St. Jude Children's Research Hospital. Our laboratory is at the forefront of landmark studies that define the genetic basis of acute leukemia, particularly acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), and develop experimental models to gain mechanistic insight into the pathogenetic basis and therapeutic intervention. The laboratory uses a broad range of approaches including genome, transcriptome, epigenome and single cell sequencing approaches, mammalian cell culture, mouse model generation using viral transduction and genome editing, functional genomic screening in vitro and in vivo using CRISPR/Cas9 genome libraries, and preclinical modeling in genetically engineered mice and patient derived xenografts. We have recently identified a novel subtype of B-cell ALL characterized by fusion of *NUTM1* (nuclear protein in testis midline carcinoma family 1) to different partner genes, including transcription factors and epigenetic regulators that drive ectopic aberrant *NUTM1* expression. By cloning and functional experimental models, we demonstrated that BRD9-NUTM1 oncoprotein sustains self-renewal and promotes the development of an aggressive leukemia in mice. We now aim to characterize the role of *NUTM1* rearrangements on transcription and chromatin states with the ultimate goal of providing valuable prognostic information and therapeutic interventions. Inhibition of BRD9 by bromodomain inhibitors or BRD9-directed chemical degraders, represents a candidate targeted therapeutic approach for patients with *BRD9-NUTM1* fusion and it will be tested in the established genetically engineered mouse models and in xenografts of patient-derived *NUTM1*-rearranged leukemia.

Skills

In this role, the student will have the opportunity to pursue research of the biology of genetic alterations in acute leukemia by using cutting-edge approaches. To succeed in this position, the student should have demonstrated proficiency in molecular biology, molecular cloning, mammalian cell culture, functional genomic screens, single cell approaches, immunoblotting, in vitro and in vivo drug screening and in vivo modeling of cancer/leukemia. Additional required skills must include strong determination and organizational skills, time management, the ability to work both independently and in a team setting, ability in troubleshooting and confidence in new approaches, work ethic and outstanding communication, writing and presentation skills.

Ogden Lab, Department of Cell & Molecular Biology, St. Jude Children's Research Hospital

The Ogden laboratory studies the Hedgehog (Hh) signal transduction pathway, which plays an evolutionarily conserved role in patterning fields of cells during embryonic development. Hh specifies cell fate decisions that result in left and right brain hemispheres, faces with one nose and two eyes and hands with one thumb and four fingers. Accordingly, mutation of Hh pathway components is causative in developmental disorders leading to brain malformation, cyclopia and digit duplications. Altered Hh signaling is also causative in pediatric cancers including medulloblastoma and rhabdomyosarcoma. As such, we are working to understand Hh signaling in healthy tissue to better understand how its function is corrupted in disease. We hope this this knowledge will lead to novel prevention and/or treatment options for developmental disorders and cancer.

The laboratory utilizes a combination of biochemical, cell biological and genetic mouse models to examine how Hh family ligands are released from ligand-producing cells and transported to activate signaling in target cells. There is a project available for a Summer Plus student to research how Hh-producing cells use specialized filopodia to transport Hh ligands across developing tissues to drive organ formation. Similar structures may be used in disease to facilitate communication between solid tumors and surrounding tumor stroma. Students will be paired with a senior lab member, so previous lab experience is not required. Upon completion of their studies, the student will have learned a set of biochemical, cell biological and genetic techniques commonly used in biomedical research.

11/16/21

The Jamy Peng lab investigates epigenetic mechanisms that regulate stem cell functions. Stem cells are responsible for originating and maintaining tissues in the human body. Over proliferation of stem cells can cause cancer, and under proliferation of stem cells can cause tissue dystrophy, immuno-deficiency, and even death. We are most interested in how stem cells make decisions to live, differentiate, or die. We study a specific pathway, named H3K27 modifications, which is causally implicated in the progression of many adult and pediatric cancers. The Rhodes Summer Plus student will work closely with Dr. Peng to evaluate how mutations in regulators of the H3K27 modifications alter stem cell survival, proliferation, and differentiation to brain organoids. If the student is more interested in mouse work, then we will work on an alternative project of examining the effect of newly discovered pathway players on the progression of a pediatric high-grade glioma. The student will join a highly enthusiastic and collaborative team and learn techniques that include culturing stem cells, mouse embryo analyses, nucleic acid and protein purification, cell sorting, gene expression profiling, immunoprecipitation, and immunofluorescence microscopy. While learning experimental techniques, past students have opted to participate in data presentations to the lab and regional conference(s).

Rosch Lab Research Project
Understanding how Tolerance Contributes to Antibiotic Resistance

Deployment of new antimicrobials is promptly circumvented by the rapid evolution of resistance, underscoring the critical need for new strategies to stay ahead in the arms-race against bacterial pathogens. It is becoming increasingly recognized that transient cell states such as antibiotic tolerance and persistence are critical drivers underlying treatment failure. These cell states represent specific populations of bacteria that are recalcitrant to antibiotic mediated-killing independent of traditional resistance mechanisms. A major gap in our knowledge is that there is a paucity of data with regards to the genetic and mechanistic basis for these cell states. These enabler cell states remain mechanistically poorly understood and seem to preferentially arise during fluctuating treatment regimens. Thereby, such cell states can drive the re-emergence of the (susceptible) bacterial infection after antibiotic pressure wanes, as well as create opportunities where multi-step high-level resistance mutations are given an extended opportunity to emerge. Therefore, because antibiotic resistant variants often follow closely on the heels of the occurrence of tolerant cell states, they can be viewed as enablers of antibiotic treatment failure and antibiotic resistance. The overall goals of the research project are to understand the full profile of possible genetic pathways that can induce antibiotic treatment failure cell states and their mechanistic consequences, treatment regimens that drive the emergence of these cell states, and how these cell states enable the emergence of antibiotic resistance.

These studies will involve working with multiple bacterial pathogens, sterile culturing techniques, colony enumeration, measuring antibiotic kill-kinetics, DNA/RNA extraction, DNA/RNA sequencing, and genetic manipulation of bacterial pathogens.

https://www.stjude.org/research/labs/rosch-lab.html

Lindsay Schwarz, Assistant Member, Developmental Neurobiology dept.

Our brains must constantly process a wide variety of stimuli from our environment to generate behavioral responses. In large part, this is regulated by a small number of norepinephrine (NE)-expressing neurons distributed throughout the brainstem, the largest group of which is the Locus Coeruleus (LC). Through release of NE, these neurons modulate numerous behaviors, including regulation of sleep/wake states, increasing attention and memory, and regulation of stress and pain response. Our lab is interested in identifying and characterizing novel mechanisms that regulate the function of NE circuits *in vivo*, towards understanding how these brain circuits help to generate distinct behaviors depending on the situation. To address these questions, we use a wide array of approaches in rodents, including viral tracing, behavioral assays, next-generation sequencing, in vivo imaging, and optogenetics.

Projects for Summer Plus students would involve working directly with current Schwarz lab members to assist with performing behavioral assays (including optogenetic or in vivo imaging methods) in rodents and histology experiments on brain tissue to visualize specific neuron populations in the brain. Students who show competency in these experiments may have the opportunity to independently develop their own project in the lab. No previous experience is required, but students must have an enthusiasm for neuroscience research and a willingness to work with animal models.

Multimodal Functional Brain Imaging and Neurorehabilitation in Pediatric Cancer

11/18/2021

St. Jude is highly motivated to develop a novel research program to acquire cognitive and psychosocial data pre- and post-cancer therapy, for characterizing and predicting the effect of cancer and cancer-therapy on sensory, perceptual, cognitive and emotional aspects of brain function and behavior, and for developing interventions with brain modulation and stimulation techniques to reverse the adverse cognitive effect of pediatric cancer. This new program would heavily rely on the application of multimodal functional brain imaging which requires highly sophisticated imaging techniques, such as functional magnetic resonance imaging (MRI), functional Near Infrared Spectroscopy (fNIRS), electro/magnetoencephalography (EEG/MEG) and brain stimulation combined with novel experimental paradigms. In addition, novel computational neuroscience and artificial intelligence methods including functional and effective connectivity, causality modeling, real-time imaging, brain-computer interfaces, and neurofeedback would be key tools for scientific investigation and translational intervention in this new research program. Rhodes College's students are invited to gain knowledge and experience in the Multimodal Functional Brain Imaging and Neurorehabilitation Hub where they can be involved in one or multiple projects in this program.

In one project, we investigate the sleep quality of the pediatric oncology population analyzing their electrophysiological signals and identifying different signatures through signal processing and artificial intelligence techniques. Rhodes college students interested will learn how to record brain signals, how to process and analyze them computationally, and how to interpret them.

In the second project, we use real-time functional magnetic resonance imaging (fMRI) to help the patients to modulate their brain activity using neurofeedback with the purpose of improving cognitive processes that may be impaired in the pediatric oncology population. Additionally, we can record activity of specific neurotransmitters that can be modulated using neurofeedback with a technique that measures magnetic resonance spectroscopy (fMRS). Rhodes college's students interested will learn how to acquire brain images in the scanner, how to process and analyze them computationally, and how to interpret them. Also, they will gain experience in real-time setups, important for neurofeedback approaches.

In the third project, we study how specific types of cancer (e.g., medulloblastoma, leukemia, sickle cell disease) affects brain cognition as well as brain related side-effects of their treatments (e.g., radiotherapy, chemotherapy, surgery). This study will involve the analysis of anatomical, functional and diffusion approaches of magnetic resonance image (MRI). During this project, the student may learn how to acquire, reconstruct, preprocess, process, segment and statistically analyze MRI data, with the opportunity of applying machine-learning computational methods to research brain pathology and therapy. Students willing to develop new computational toolboxes to automatically perform the mentioned steps are also welcome.

Additionally, our group is interested in exploring focused ultrasound stimulation to modulate neural activity in specific brain regions with the goal of improving functioning on cognitive tasks. This work is still in the preliminary development stage, and Rhodes students could help us develop computer models of the spatial extent, precision, and intensity of ultrasound stimulation using CT scans of rodent and human brains. This work would help inform experimental planning and better identify stimulation parameters and targets for ultrasound stimulation.

In summary, the new research program described above has several opportunities for research and technical development in several cross-disciplinary areas including medical imaging, artificial intelligence, experimental neuroscience and neurorehabilitation, involving in which would benefit the Rhodes student in their future career. Those interested could refer to the following contact information.

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Rhodes Summer Plus

Willard Lab – Social-Emotional Development in Youth with Cancer

Victoria W. Willard, PhD
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Department of Psychology

Some children with cancer may experience difficulty making friends and interacting with peers post-treatment. These difficulties are most often seen in children who were treated for brain tumors, and it is thought that the neurotoxic treatments that were required to treat their disease cause cognitive problems (like poor attention and slow processing speed) that impact their ability to participate socially. My lab focuses on identifying and quantifying the social difficulties that youth with brain tumors, and those with other forms of childhood cancer, experience in an effort to eventually design interventions that will improve their functioning. Active projects are focused on school-aged survivors of brain tumors, preschool-aged children with brain tumors and non-CNS solid tumors, and adolescents and young adults with all cancer diagnoses. A student could be involved in all active projects, including tasks such as observation of study visits, data entry, medical chart review, data analysis, and data presentation (conference posters/presentations and manuscripts). It would be helpful if the student has completed Psychology courses focused on developmental psychology, research methods, and statistics. Students who have experience with and are comfortable with children would be an asset.

Lab of Gang Wu
Center for Applied Bioinformatics

Machine learning based methods for sequence variant calling and filtering

Many rare heritable diseases or cancers are caused by rare mutations in DNA sequence. Whole exome and genome sequencing are high throughput technologies that can output almost all DNA sequences in the exome region or whole genome regions. When the output sequences are aligned to a reference genome, rare variants/mutations can be identified. To achieve accurate and powerful identification of causal variants, accurate variant calling from sequencing data is vital. Machine learning methods, such as VQSR in the tool GATK using Gaussian mixture models, have been developed for variant filtering after being called by GATK. Recently, another machine learning based method called DeepVariant shows even better performance. It makes use of recent advances in deep learning which is an end-to-end learning method and can achieve superior performance when there are huge training data sets. In St Jude's Center for Applied Bioinformatics, we have accumulated a large number of whole exome and genome data, such as those from PCGP and St Jude LIFE cohorts. These provide us a good opportunity to evaluate different variant calling and filtering methods and to develop novel methods if there is a need. In this project, we will explore and develop different strategies in applying machine learning methods for variant calling and filtering. Students need to have good programming skills, such as Python, C++ or Java. Some understanding of machine learning methods or related machine learning libraries is ideal but not necessary. Students will learn the basic concept of machine learning, gain experiences in applying the state-of-the-art machine learning methods, and may develop novel applications of machine learning methods in variant QC and filtering.

Jun Yang, MBBS, MS, PhD (Assistant Member), Surgery Dept., Developmental Biology & Solid Tumor Program.

Dr. Yang's research program focuses on drugging the genetic and epigenetic vulnerability of cancers, which is supported by American Cancer Society and National Cancer Institute. With sophisticated cutting-edge epigenetic approaches and novel selective small molecules, Dr. Yang's laboratory is currently exploring the efficacy and investigating the mechanism of targeted therapy by using transgenic neuroblastoma, liver and rhabdomyosarcoma models and patient-derived xenograft models.

We recently identified a new mechanism of RNA splicing that plays an essential role in maintaining cancer cell survival. By using a new class of drug, called "molecular glue" that bridges the RNA splicing factor and the ubiquitin system for protein degradation, we are able to completely cure the mice that bear tumors that even exceeded 15% of body weight (https://www.science.org/doi/10.1126/sciadv.abj5405). This project will ask (1) What mechanisms are responsible for the efficacy and resistance to "molecular glue"; (2) Can combination of "molecular glue" with immunotherapy be a superior anticancer approach? This project involves techniques and skills including genomic editing with CRISPR, gene knockdown by shRNA, retroviral and lentiviral based gene delivery, RNA-sequencing, epigenomic approaches, bioinformatics analysis, Western blot, RT-PCR, in vivo disease models, etc.

The trainees will be taught in an environment where scientists and clinicians have close collaborations, are expected to learn the knowledge of modern cancer biology and master new techniques, eventually be able to perform project independently. Basic training including knowledge in biology and biochemistry and cell culture is a prerequisite.