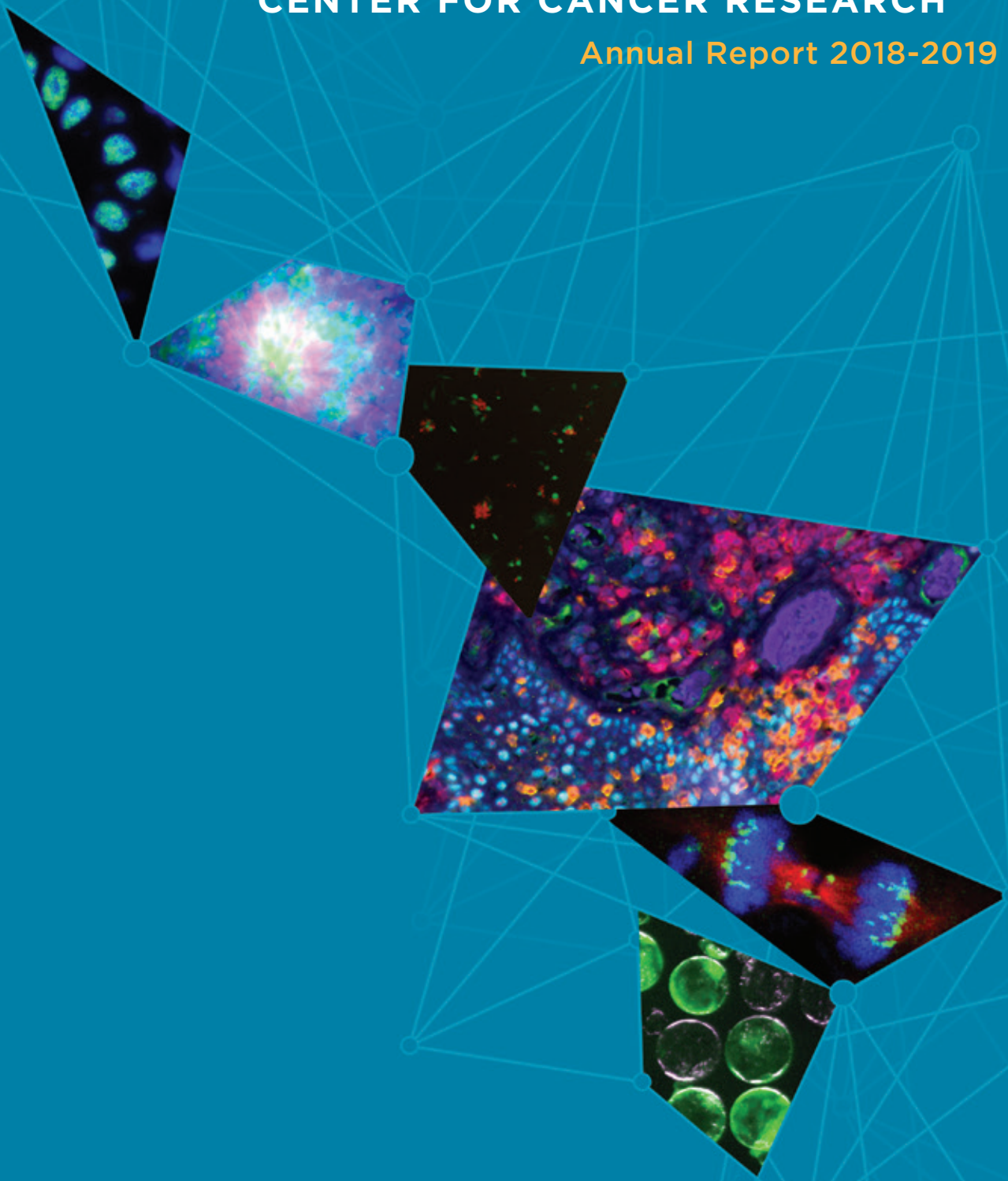
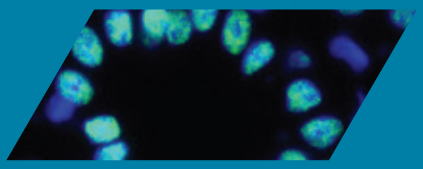
CENTER FOR CANCER RESEARCH

Annual Report 2018-2019



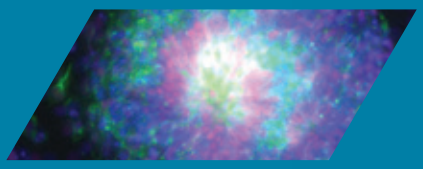
MASSACHUSETTS GENERAL HOSPITAL
CANCER CENTER



Light blue staining shows activated STAT5 in luminal mammary epithelial cells, which is required for terminal differentiation, and the absence of which may be an early step in breast cancer progression.

Image created by Nicole Forster PhD

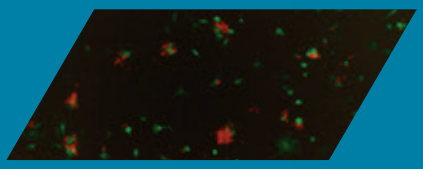
Ellisen Laboratory



The image is an Immunofluorescence image from induced pluripotent stem (iPS) cells derived from a human patient with an homozygous mutation in the chromatin factor SIRT6. The iPS cells were forced to differentiate into neural progenitors cells, and stained with a neural marker (Nestin-green) and an early embryo marker (Sox2-red). Note that Sox2 is still expressed in these cells (normal NPCs silenced Sox2 at this stage).

Image Credit: Christina Ferrer

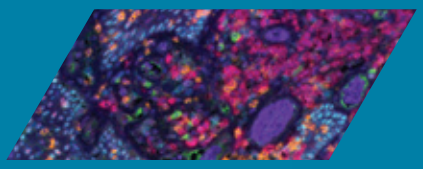
Motoslavsky Laboratory



Glioblastoma tumor cells (green) by surrounded and lysed by CAR T cells (red).

Image acquired by Ana Castano, MD in Maus lab.

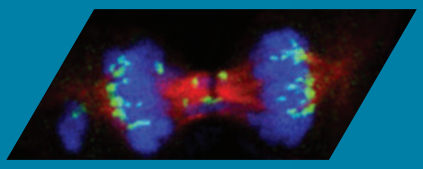
Maus Laboratory



Multispectral image of a tumor specimen from a head and neck patient.

Image courtesy of Joao Oliveira Da Costa, PhD

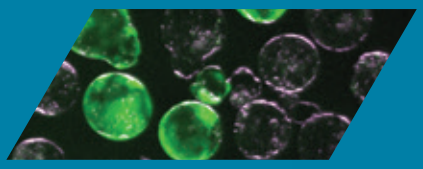
Stott Laboratory



Indirect immunofluorescence image of a human RPE-1 exiting mitosis with several missegregation events. Cell was stained for microtubules (red), centromeres (green) and DNA (blue).

Image credit: Lilian Kabeche, PhD

Zou Laboratory



Use of X chromosome reporter to isolate male and female blastocysts for subsequent methylation analysis. Depicted are blastocysts from a cross between male mice carrying an X-linked GFP reporter and wild-type female mice. GFP-positive blastocysts are female while GFP-negative blastocysts are male.

Image credit: Jiho Choi

Hochedlinger Laboratory

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Reports from the Principal Investigators

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Jonathan Kraft Prize for Excellence in Cancer Research

Presented by the Massachusetts General Hospital Cancer Center

2019

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Professor in Immunotherapy
Director, Center for Cellular Immunotherapies
University of Pennsylvania Perelman School of Medicine

2018

Charles Swanton, MD, PhD
Professor and Chair, Personalized Cancer Medicine
University College London Cancer Institute, London, UK

2017

Kevan M. Shokat, PhD
Professor and Chair, Department of Cellular and Molecular Pharmacology, UCSF

Professor, Department of Chemistry, UC Berkeley

2016

Joan A. Steitz, PhD
Sterling Professor of Molecular Biophysics and Biochemistry, Yale School of Medicine

2015

C. David Allis, MD, PhD
Joy and Jack Fishman Professor, Laboratory of Chromatin Biology and Epigenetics, Rockefeller University

The Annual MGH Award in Cancer Research

In memory of Nathan and Grace Shiff

2014

Hans Clevers, MD, PhD
President of the Royal Netherlands Academy of Arts and Sciences
Professor of Molecular Genetics
University Utrecht, Netherlands

2013

James Allison, PhD
Chair, Department of Immunology
MD Anderson Cancer Center, Houston, Texas

2012

Craig Thompson, MD
President and Chief Executive Officer
Memorial Sloan-Kettering Cancer Center, New York

2011

Michael Stratton, MD, FRS
Director, Wellcome Trust Sanger Institute, Cambridge, UK

2010

Charles Sawyers, MD
Chairman of the Human Oncology and Pathogenesis Program
Memorial Sloan-Kettering Cancer Center, New York

2009

Bert Vogelstein, MD
Director of the Ludwig Center for Cancer Genetics & Therapeutics
Sidney Kimmel Comprehensive Cancer Center
Johns Hopkins University, Maryland

2008

Titia de Lange, PhD
Associate Director of the Anderson Cancer Center
Rockefeller University, New York

2007

Joan Massague, PhD
Chairman of the Cancer Biology and Genetics Program
Memorial Sloan-Kettering Cancer Center, New York

2006

Anton Berns, PhD
Director of Research and Chairman of the Board of Directors,
Netherlands Cancer Institute and Antoni van Leewenhoek Hospital
Netherlands

Center for Cancer Research Faculty

Daniel A. Haber, MD, PhD
Director, Massachusetts General Hospital Cancer Center
Kurt J. Isselbacher Professor of Oncology

Kurt J. Isselbacher, MD
Director Emeritus, Massachusetts General Hospital Cancer Center
Mallinckrodt Distinguished Professor of Medicine

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Professor of Medicine

Lee Zou, PhD
Associate Scientific Director
James and Patricia Poitras Chair in Cancer Research
Professor of Pathology

Charlestown Laboratories

*Martin Aryee, PhD**
Assistant Professor of Pathology

Liron Bar-Peled, PhD
Faculty Member†

Cyril Benes, PhD
Assistant Professor of Medicine

Priscilla Brastianos, MD
Assistant Professor of Medicine

Mark Cobbold, MRCP, PhD
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Assistant Professor of Medicine

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*Assistant Professor in Dermatology^*

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Assistant Professor of Radiation Oncology

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Assistant Professor of Medicine

Nir Hacohen, PhD
Professor of Medicine

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Assistant Professor of Medicine•

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Associate Professor of Medicine

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Professor of Pathology

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Andrea I. McClatchey, PhD
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Alexandra-Chloé Villani, PhD
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Professor of Pathology

Simches Laboratories

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*Konrad Hochedlinger, PhD***
Professor of Medicine

*Hanno Hock, MD, PhD***
Assistant Professor of Medicine

Raul Mostoslavsky, MD, PhD
Associate Professor of Medicine

David Sweetser, MD, PhD
Assistant Professor of Pediatrics□

Shobha Vasudevan, PhD
Assistant Professor of Medicine

* Joint appointment, Massachusetts General Hospital Cancer Center and Molecular Pathology Unit

** Joint appointment, Massachusetts General Hospital Cancer Center and Center for Regenerative Medicine and Technology

^ Joint appointment with MGH Cutaneous Biology Research Center

Joint appointment with MGH Molecular Radiation Oncology Unit

• Joint appointment with MGH Transplantation Research Center

◊ Joint appointment with Ragon Institute of Harvard and MIT

□ Joint appointment with MGH Pediatric Hematology Oncology Unit

† Appointment process initiated

Reports from the Principal Investigators

Martin Aryee, PhD

The Aryee laboratory develops analysis methods for studying the genetic and epigenetic basis of cancer and other diseases. Most of their work is focused on improving our understanding of how aberrations in the physical and chemical structure of DNA within the nucleus is linked to cancer and other common diseases. Projects range from basic biology, probing how DNA misfolds in cancer cells, to clinical applications aiming to develop blood tests for early detection of cancer. The lab also develops tools that aim to enable the safe translation of gene editing techniques such as CRISPR into human therapeutics.

Aryee Laboratory

Martin Aryee, PhD

Divy Kangeyan*

Caleb Lareau*

Kelly Mosesso*

Ayush Raman, PhD

Alejandro Reyes, PhD

* PhD Candidate

Tumor heterogeneity

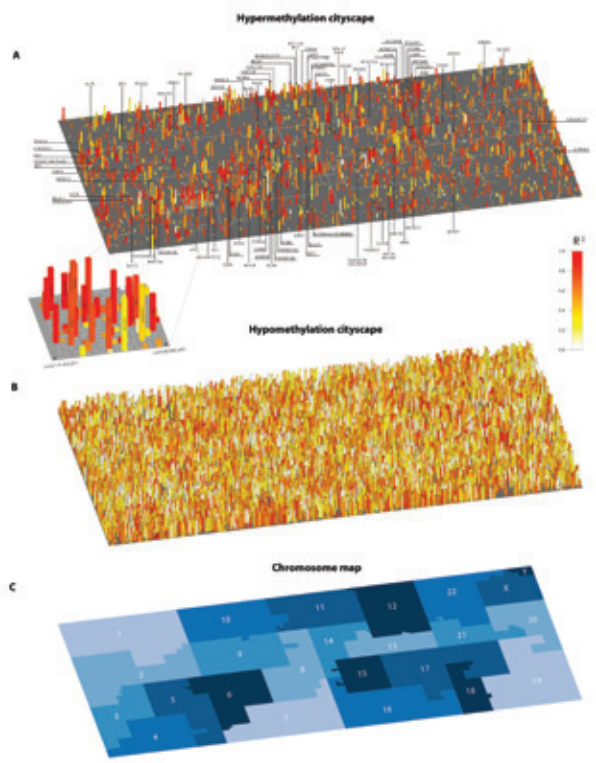
We develop statistical methods to improve our understanding of cell-to-cell variability and its relationship to cancer-related phenotypes. Much of this work relates to the computational and statistical challenges posed by single-cell transcriptome and epigenome data. The goal of these methods is to characterize the somatic changes that occur during tumor development and that are ultimately responsible for disease progression and resistance to therapy.

Different tumors, even of the same type, can harbor extremely heterogeneous epigenetic alterations. To investigate the role of epigenetic stochasticity in cancer, we recently applied a statistical model to study patterns of inter- and intra-individual tumor heterogeneity during metastasis. We established that metastatic prostate cancer patients develop distinctly unique DNA methylation signatures that are subsequently maintained across metastatic dissemination. Further, by quantifying the stability of these individualized DNA methylation profiles we showed that they were strikingly similar to that of copy number alterations, a finding with implications for the promise of epigenetic alterations as diagnostic and therapeutic targets in cancer.

Epigenome mapping

Unlike genome sequencing which has well established experimental and analytical protocols, epigenome mapping strategies are still in their infancy and, like other high-throughput techniques, are plagued by technical artifacts. A central theme of our research involves the development of methods for extracting signal from noisy high-throughput genomic assays. The goal of such preprocessing methods is to transform raw data from high-throughput assays into reliable measures of the underlying biological process.

Until recently, studies of DNA methylation in cancer had focused almost exclusively on CpG dense regions in gene promoters. We helped develop the statistical tools used to analyze the first genome-scale DNA methylation assays designed without bias towards CpG islands. These tools enabled the discovery that the majority of both tissue-specific and cancer-associated variation occurs in regions outside of CpG islands. We showed that there is a strong overlap between genomic regions involved in normal tissue differentiation, reprogramming during induced pluripotency, and cancer.



DNA methylation "Cityscape" plots of lethal metastatic prostate cancer highlight inter-tumor epigenetic heterogeneity.

Genomic cityscapes of somatic (A) hypermethylation and (B) hypomethylation. Each chromosome is folded into neighborhoods as shown in (C). Each structure represents a genomic region showing a somatic methylation alteration. The height of each structure indicates the number of tumors showing an alteration at this site. The color scale represents the degree of stability of these alterations across metastases within individuals. The magnified region in (A) illustrates a representative chromosomal segment showing clustering of frequently hypermethylated regions (skyscrapers).

Epigenomic studies of complex disease

Despite the discovery of numerous disease-associated genetic variants, the majority of phenotypic variance remains unexplained for most diseases, suggesting that non-genetic factors play a significant role. Part of the explanation will lie in a better understanding of epigenetic mechanisms. These mechanisms are influenced by both genetic and environmental effects and, as downstream effectors of these factors, may be more directly related to phenotype. There is hope that epigenetic alterations may provide therapeutic targets for pharmacological intervention, due to their reversible nature. However, the broad extent of epigenetic dysregulation in cancer and many other diseases complicates the search for the small subset of alterations with a causal role in pathogenesis. We are developing computational methods to integrate genome-wide genetic and epigenetic data with the goal of identifying the subset of functionally important epigenetic alterations.

Selected Publications:

Akcakaya P, Bobbin ML, Guo JA, Malagon-Lopez J, Clement K, Garcia SP, Fellows MD, Porritt MJ, Firth MA, Carreras A, Baccega T, Seeliger F, Bjursell M, Tsai SQ, Nguyen NT, Nitsch R, Mayr LM, Pinello L, Bohlooly-Y M, **Aryee MJ**, Maresca M, Joung JK. In vivo CRISPR editing with no detectable genome-wide off-target mutations. *Nature*. 2018 Sep;561(7723):416-419

Lareau CA, **Aryee MJ**. hichipper: a preprocessing pipeline for calling DNA loops from HiChIP data. *Nat Methods*. 2018 Feb 28;15(3):155-156. doi: 10.1038/nmeth.4583. No abstract available.

Lareau CA, **Aryee MJ**. diffloop: a computational framework for identifying and analyzing differential DNA loops from sequencing data. *Bioinformatics*. 2018 Feb 15;34(4):672-674.

Kleinstiver BP, Prew MS, Tsai SQ, Topkar VV, Nguyen NT, Zheng Z, Gonzales AP, Li Z, Peterson RT, Yeh JR, **Aryee MJ**, Joung JK. Engineered CRISPR-Cas9 nucleases with altered PAM specificities. *Nature*. 2015 Jul 23;523(7561):481-5.

Ziller MJ, Hansen KD, Meissner A, **Aryee MJ**. Coverage recommendations for methylation analysis by whole-genome bisulfite sequencing. *Nat Methods*. 2015 Mar;12(3):230-2.

Aryee MJ, Jaffe AE, Corrada-Bravo H, Ladd-Acosta C, Feinberg AP, Hansen KD, Irizarry RA. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. *Bioinformatics*. 2014 May 15;30(10):1363-9.

Liron Bar-Peled, PhD

Research in **the Bar-Peled laboratory** sits at the interface of cellular metabolism and signal transduction and focuses on understanding how cancer cells respond to altered metabolic states. Rapidly proliferating cancer cells are characterized by increased production of toxic metabolic byproducts known as reactive oxygen species (ROS) that at high levels potently block cancer cell growth. To neutralize high ROS levels, cancer cells activate the NRF2 pathway, which governs the cellular antioxidant response. While the NRF2 pathway is critical for cancer growth, the molecular mechanisms by which this pathway functions and provides cancers cells with a proliferative advantage remain poorly understood. By combining frontier molecular, chemical and proteomic approaches, research in our lab has revealed that NRF2 establishes a unique cellular environment that protects critical proteins required for cancer cell growth from inactivation by ROS. Our studies indicate that these ROS-regulated proteins are highly targetable by small molecule inhibitors allowing us to develop chemical tools to inactivate these dependencies in cancers.

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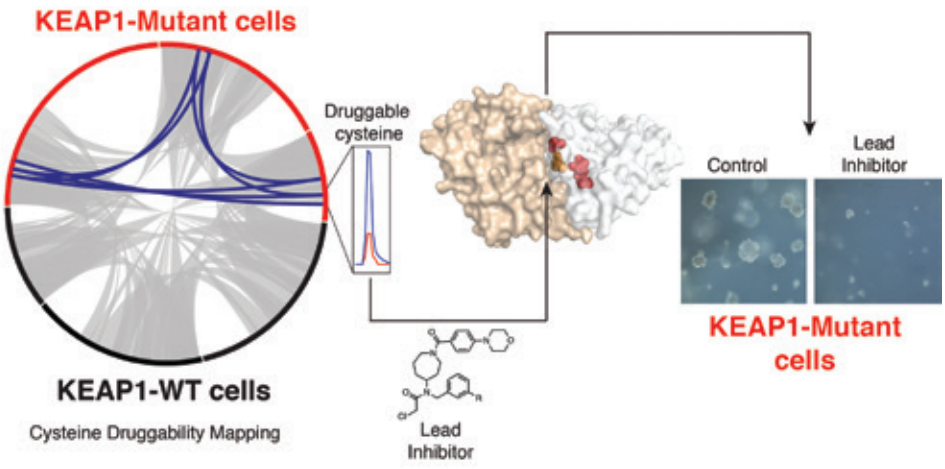
Bar-Peled Laboratory

(opens early 2019)
Liron Bar-Peled, PhD

Cancer cells display remarkable plasticity allowing them to adapt to ever changing environments. A key feature of this plasticity is their ability to rewire core metabolic networks to provide a steady source of energy and building blocks needed for rapid growth. This demand for energy produces byproducts including ROS that alters the function of proteins, DNA and lipids, and if left unchecked, results in oxidative stress and impairs cancer cell viability. To counter a rise in oxidative stress, cells activate the NRF2 transcription factor leading to the expression of a vast network of antioxidant and detoxification genes that restore redox homeostasis. Multiple cancer cells, including ~30% of non-small cell lung cancers (NSCLCs) activate NRF2 through the genetic disruption of its negative regulator KEAP1. Despite its clear importance in cancer cell proliferation, we know remarkably little about how the NRF2/KEAP1 pathway functions within cancer cells or how ROS modification of proteins alters their function. Our long-term goal is to understand how cancer cells sense and respond to ROS and to pharmacologically modulate these pathways in cancers where they are deregulated.

Redox control pathways in Lung Cancer

Our recent studies focus on how the intracellular environment generated by NRF2 in NSCLCs is required for cancer cell proliferation. By employing a chemical proteomics platform (isoTOP-ABPP) that identifies changes in cysteine reactivity mediated by ROS, we demonstrated that NRF2 is required for the protection of dozens of proteins from ROS modification. We found that silencing NRF2 in NSCLCs reduced the reactivity of the catalytic cysteine of the glycolytic enzyme GAPDH without changing GAPDH protein abundance. Concomitant knockdown of NRF2 significantly reduced



(Left) A cysteine druggability map identifies proteins exclusively druggable in KEAP1-mutant NSCLC cells enabling the development of small molecule inhibitors that disrupt NR0B1 protein interactions (middle) and block KEAP1-mutant cell growth (right). Images from Bar-Peled et al., 2017.

GAPDH enzyme activity and glycolytic flux, a metabolic pathway required to fuel cancer cell proliferation. These results illustrate how NRF2 can regulate enzyme and pathway activity, not through direct transcriptional control, but rather by fostering a favorable redox environment required for proper enzyme function. Current studies in our lab seek to elucidate how other proteins are post-translationally regulated by NRF2 and feedback into this pathway. To address these questions, we are studying the function of ROS-regulated sites on proteins as well as the identifying reactive metabolites that modify them.

Druggable co-dependencies

Our investigations suggest that the cellular state created by NRF2 may be exploited to develop inhibitors targeting proteins whose expression and function are stimulated by this environment. Because of their importance to protein function, cysteines are targeted by multiple clinically approved inhibitors. To identify pharmacological targets of the NRF2 pathway, we use powerful chemical proteomic platforms (cysteine druggability mapping) to identify the landscape of protein druggability (e.g. ligand-protein interactions) in genetically defined lung cancers. Our studies reveal that multiple proteins, including the orphan nuclear receptor NR0B1, are exclusively druggable in KEAP1-mutant, NRF2-activated cells. By developing a small molecule inhibitor that disrupts NR0B1 protein interactions we show that NR0B1 functions as a critical signaling node within the NRF2 pathway to support its pro-proliferative transcriptional output required for anchorage-independent growth. Recently we uncovered that cysteine residues that are sensitive to ROS modification are highly targetable by covalent inhibitors. Our current studies suggest that these sites may be exploited to develop inhibitors that target proteins required for the proliferation of NRF2-activated cancers.

Ongoing projects:

1. Determine how cancer proteomes respond to changes in the intracellular redox environment
2. Elucidate the role of NRF2-regulated reactive metabolites on protein function
3. Decipher how cells adapt to anchorage-independent growth
4. Identify druggable transcriptional dependencies in genetically-defined cancers

Selected Publications:

Bar-Peled L*†, Kemper EK*, Suciu RM, Vinogradova EV, Backus KM, Horning BD, Paul TA, Ichu TA, Svensson RU, Olucha J, Chang MW, Kok BP, Zhu Z, Ihle N, Dix MM, Hayward M, Jiang P, Saez E, Shaw RJ, and Cravatt BF.† (2017). Chemical Proteomics Identifies Druggable Vulnerabilities in a Genetically Defined Cancer. *Cell*. 171: 696-709.

Wang S, Tsun ZY, Wolfson RW, Shen K, Wyant GA, Plovanich ME, Yuan ED, Jones T D, Chantranupong L, Comb W, Wang T, **Bar-Peled L**, Zoncu R, Straub C, Kim C, Park J, Sabatini BL, and Sabatini DM. (2015) The amino acid transporter SLC38A9 is a key component of a lysosomal membrane complex that signals arginine sufficiency to mTORC1. *Science*. 347: 188-194.

Bar-Peled L. (2014). Size does matter. *Science* 346: 1191-1192.

Tsun ZY, **Bar-Peled L***, Chantranupong L*, Zoncu R, Wang T, Kim C, Spooner E., Sabatini DM. (2013). The Folliculin Tumor Suppressor is a GAP for the RagC/D GTPases That Signal Amino Acid Levels to mTORC1. *Molecular Cell* 52: 495-505.

Bar-Peled L*, Chantranupong L*, Cherniack AD, Chen WW, Ottina KA, Grabiner BC, Spear ED, Carter SL, Meyerson ML, and Sabatini DM. (2013). A tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. *Science* 340: 1100-1106.

Bar-Peled L., Schweitzer LD, Zoncu R., and Sabatini DM. Ragulator is a GEF for the Rag GTPases that signal amino acid levels to mTORC1 (2012). Cell 150: 1196-1208.

*These authors contributed equally to this work

†Co-corresponding authors

Nabeel Bardeesy, PhD

Pancreatic cancer and biliary cancer are among the most lethal types of human cancers. **The Bardeesy laboratory** has developed a series of genetically engineered mouse models to define the role of key gene mutations that drive these cancer types. Current projects focus on defining roles for cancer genes in controlling the way cells modulate their growth and utilize energy in response to available nutrients, and on identifying epigenetic regulators responsible for changes in cellular differentiation state that lead to cancer initiation and maintenance.

• • •

Bardeesy Laboratory

Nabeel Bardeesy, PhD
Antoine Escudier
Leah Liu, PhD
Krushna Patra, PhD
Vu Phuong, BSc
Lei Shi, PhD
Krishna Tummala, PhD
Vajira Weerasekara, PhD
Jia Chi Yeo, PhD

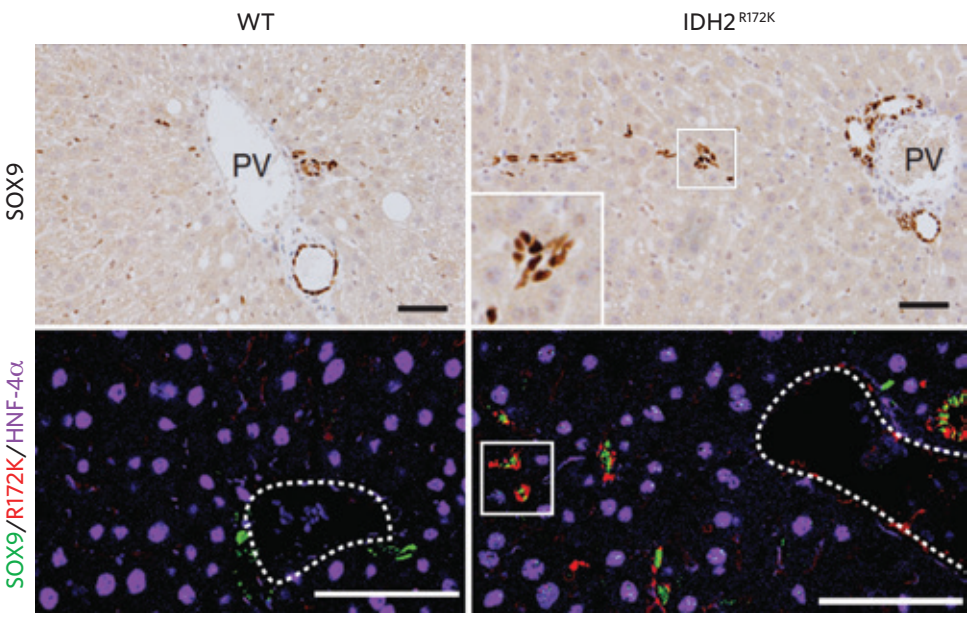
The Bardeesy lab focuses on defining the pathways driving the pathogenesis of pancreatic and biliary cancers. Our lab has developed a series of genetically engineered mouse models that has elucidated the functional interactions of major gene mutations associated with these diseases in humans. Specifically, we have characterized the roles of key cancer genes in the control of cellular differentiation states and in metabolic regulation.

Interplay between metabolism and chromatin regulation in pancreatic and biliary cancer

An important area of current focus in our lab is to elucidate the epigenetic regulators of pancreatic cancer and biliary cancers, with particular attention paid to factors that subvert normal differentiation pathways and reprogram cancer cell metabolism. In pancreatic cancer, we have linked mutations in LKB1/STK11 and other important genetic alterations to changes in metabolism that ultimately alter epigenetic states. Identifying these pathways have provided insights in mechanisms of cell transformation arising from these mutations and predict novel therapeutic vulnerabilities. In biliary cancer, there are recurrent mutations in the IDH1 and IDH2 genes. Mutant IDH proteins acquire a novel enzymatic activity allowing them to convert alpha-ketoglutarate (αKG) to 2-hydroxyglutarate (2HG), which inhibits the activity of multiple αKG-dependent dioxygenases, including the JmjC family histone demethylases. We are focusing on how IDH mutations affect epigenetic programs and regulation of cellular identity in the liver.

Targeting master regulators of metabolic reprogramming in PDAC

In order to couple rapid growth with available nutrients, cancers employ profoundly altered networks of biosynthetic and catabolic pathways. This requirement for metabolic reprogramming is particularly acute in PDAC, which is characterized by hypoxia and limited nutrient availability, and activates anti-oxidant gene expression and autophagy (cellular self-catabolism) as necessary adaptive metabolic changes. While these pathways offer attractive new therapeutic targets, the underlying mechanisms driving altered PDAC metabolism are unclear. We have focused on identifying master transcriptional regulators that broadly orchestrate metabolic reprogramming in PDAC.



Mutant IDH causes expansion and impaired differentiation of liver progenitor cells leading to biliary cancer. Immunohistochemistry (top) and immunofluorescence (bottom) of livers from wild type (WT) and transgenic mice expressing mutant IDH2R172K. Sox9 (top, brown stain; bottom, green) normally marks bile duct cells adjacent to the portal vein (PV), whereas there is aberrant accumulation of Sox9-expressing cells progenitor/stem cells in IDH mutant livers. These cells are highly prone to progression to biliary cancer (cholangiocarcinoma). Image from Saha, Parachoniak et al., Nature 2014.

Mouse models of biliary cancer

Recent genetic studies have identified multiple recurrent mutations in biliary cancers and have indicated considerable genetic heterogeneity between individual tumors. A key limitation in the field includes a paucity of experimental systems with which to define the contributions of the lesions to biliary cancer progression. We have established a series of genetically engineered mouse models that incorporate combinations of the major mutations found in the human disease. In addition, our ongoing efforts include the development of a human biliary cancer cell line bank for the use of genetic and small-molecule, screening in genetically defined subtypes of this cancer.

Control of liver progenitor cells and biliary cancer development

The Hippo pathway is a conserved regulator of organ size. Our lab has shown that this pathway is central for controlling the quiescence of liver progenitor cells, and that its loss leads to massive liver overgrowth and development of both major types of liver cancer (hepatocellular carcinoma and cholangiocarcinoma). The lab is studying the circuitry of the Hippo pathway in liver progenitor cells and the key mediators of tumorigenesis found downstream of this pathway.

Selected Publications:

Patra KC, Kato Y, Mizukami Y, Widholz S, Boukhali M, Revenco I, Grossman EA, Ji F, Sadreyev RI, Liss AS, Screaton RA, Sakamoto K, Ryan DP, Mino-Kenudson M, Castillo CF, Nomura DK, Haas W, **Bardeesy N**. Mutant GNAS drives pancreatic tumourigenesis by inducing PKA-mediated SIK suppression and reprogramming lipid metabolism. *Nat Cell Biol*. 2018 Jul;20(7):811-822.

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Fitamant J, Kottakis F, Benhamouche S, Tian H, Chuvin N, Parachoniak CA, Nagle J, Perera RM, Deshpande V, Zhu AX, Lai A, Min B, Hoshida Y, Avruch J, Sia D, Camprecios G, McClatchey AI, Llovet JM, Morrissey D, Raj L, **Bardeesy N***. YAP inhibition restores hepatocyte differentiation in advanced HCC leading to tumor regression. *Cell Rep.* 2015 Mar 10. pii: S2211-1247(15)00173-4.

*Co-corresponding authors

Cyril Benes, PhD

The Benes laboratory, also known as The Center for Molecular Therapeutics, is engaged in the design and application of personalized therapies for cancer. Targeted cancer treatments have emerged from research studies showing that the biology of cancer cells differs from that of healthy cells, and that each person's cancer has a unique genetic signature. Our goal is to pinpoint the cancer cells' biological weak points and then to attack those weak points with smart drugs that are specifically designed for such an attack. We use a very large collection of previously established tumor cell lines derived from many different cancers as well as newly established lines from patients treated at MGH. We are focused on developing molecular diagnostics that will reveal the best treatment course for each patient, and on discovering how gene mutations in cancer can be exploited to develop new treatments.

• • •

Benes Laboratory

Nathaniel Adams
Cyril Benes, PhD
Eliane Cortez, PhD
Farideh Davoudi, PhD
Kristin Dionne
Regina Egan
Samar Ghorbanpoor, PhD
Patricia Greninger
Patricia Hare
Haichuan Hu, MD
Eunice Kim
Ellen MurchieJulie Nasuta
Xunqin "Elizabeth" Yin

We are studying the molecular basis of response to anticancer agents.

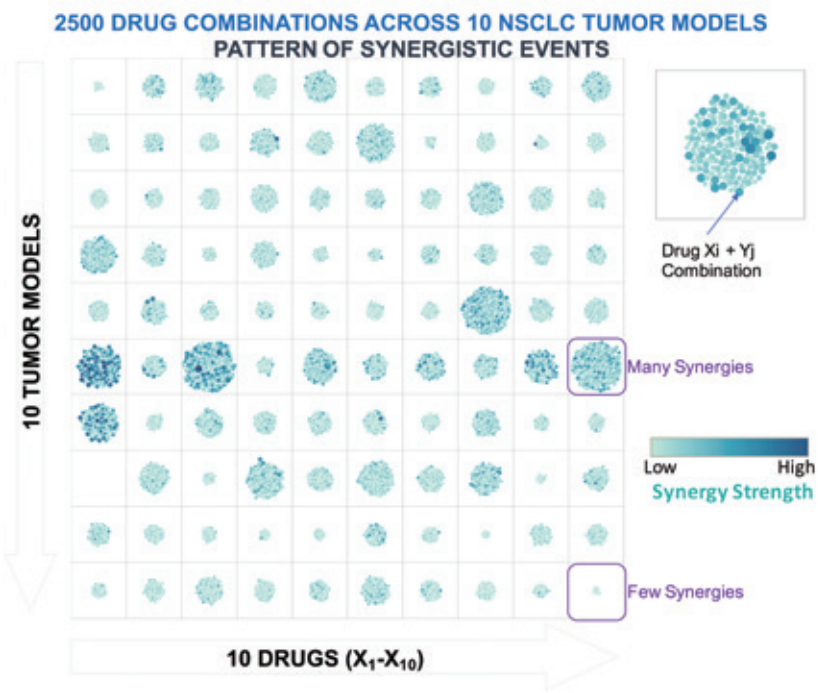
Genetics of Cancer Therapeutic Response

Clinical responses to anticancer therapeutics are often restricted to a subset of cases treated. In some instances, clear evidence is available that correlates clinical responses with specific tumor genotypes. Our goal is to identify tumor cell states (i.e., genotypes, gene expression) that predict sensitivity to anticancer agents. To accomplish this goal, we use high-throughput screening and expose 1,000 cell lines derived from a broad spectrum of cancers to known and potential anticancer therapeutic agents. We characterize the activity of single agents and combinations to discover therapeutic applications and biomarkers of response that could be used to select patients most likely to benefit.

The use of a very large cell line collection allows us to capture some mutational events that—although relatively rare—are very important for therapeutic response. In addition, while some patient selection strategies have proven quite successful, a wide range of variation in response to treatment exists in almost all cases. Similar to this clinical observation—and perhaps related mechanistically—our large cell line collection allows us to observe important variation in drug response within a given sensitizing genotype. For example, among BRAF-mutant cell lines which are, as a group, remarkably sensitive to BRAF inhibitors, some lines do not respond significantly. Based on these observations, we aim to identify additional biomarkers that will permit more accurate prediction of drug response in the clinic.

Resistance to Cancer Therapies

Even for the most successful anticancer therapies, drug resistance invariably emerges and limits the impact on patient lives. The molecular mechanisms underlying acquired resistance to cancer therapeutics are not well defined but are likely to be different for each therapy and cancer. We are investigating how drug combinations could overcome resistance, and within this context, studying how changes in intracellular signaling pathways affect drug response.



Identification of synergistic drug combinations across non-small cell lung cancer (NCLC) models. 2,500 Drug combinations were tested across 10 different tumor derived models. In each model a minority of the tested combinations are synergistic. Across models a given drug combination can be synergistic or not. The counts and strength of synergies are shown in the grid. Each dot shows the synergy score for a given combination in a given tumor model (zoomed up panel on right).

We are tackling the problem of therapeutic resistance using cell lines made resistant in the laboratory or isolated from resistant tumors. Previous results have shown that these cell line models do recapitulate at least some of the mechanisms of resistance at play in patients. We interrogate combinations of a panel of clinically relevant anticancer drugs as a way to quickly identify candidate therapeutic strategies and to jumpstart mechanistic studies that will help characterize the molecular basis of acquired resistance. To complement genomic guided therapeutic decisions we are developing approaches to rapidly grow cells from tumor and identify clinically relevant drugs with potential for clinical efficacy in the patients from which the cells were obtained.

In recent studies we have explored the role of cells present in the tumor together with the cancer cells. Tumors contain fibroblasts, endothelial cells and immune cells among others. We are studying the impact that the fibroblasts in the tumor have on response to therapy. We use biopsy derived fibroblasts and cancer cells to study their relationship and understand how fibroblasts might provide cancer cells with some protection against drug treatment.

We are also tackling the problem of resistance using a very different and complementary approach. We systematically identify genes that can cause resistance to a particular drug in a given context using a transposon-based genetic screen. Transposons are mobile genetic elements that can insert into a host genome—in our case, the genome of cancer cells. We use an engineered version of a transposon so we can control its mobility and identify genes with expressions that are modified by its insertion, leading to drug resistance.

Selected Publications:

Lochmann TL, Powell KM, Ham J, Floros KV, Heisey DAR, Kurupi RIJ, Calbert ML, Ghotra MS, Greninger P, Dozmorov M, Gowda M, Souers AJ, Reynolds CP, **Benes CH**, Faber AC. Targeted inhibition of histone H3K27 demethylation is effective in high-risk neuroblastoma. *Sci Transl Med.* 2018 May 16;10(441).

Dardaei L, Wang HQ, Singh M, Fordjour P, Shaw KX, et al. SHP2 inhibition restores sensitivity in ALK-rearranged non-small-cell lung cancer resistant to ALK inhibitors. *Nat Med*. 2018 May;24(4):512-517.

Yuan TL, Amzallag A, Bagni R, Yi M, Afghani S, Burgan W, et al. Differential Effector Engagement by Oncogenic KRAS. *Cell Rep*. 2018 Feb 13;22(7):1889-1902.

Kodack DP, Farago AF, Dastur A, Held MA, Dardaei L, Friboulet L, von Flotow F, et al. Primary Patient-Derived Cancer Cells and Their Potential for Personalized Cancer Patient Care. *Cell Rep*. 2017 Dec 12;21(11):3298-3309.

Lapek JD Jr, Greninger P, Morris R, Amzallag A, Pruteanu-Malinici I, **Benes CH**, Haas W. Detection of dysregulated protein-association networks by high-throughput proteomics predicts cancer vulnerabilities. *Nat Biotechnol*. 2017 Oct;35(10):983-989.

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Bradley Bernstein, MD, PhD

The Bernstein laboratory studies how the DNA in the human genome is packaged by a structure called chromatin. A central question in human biology is how the one genome we inherit at birth can give rise to the hundreds of cell types in the body. The genome consists of genes that code for the protein machines in our cells as well as regulatory elements that control those genes. A liver cell is different from an immune cell or a neuron because it makes different proteins. The way a gene is organized into chromatin predicts whether it will be turned on or off—and thus make protein—in a particular cell type. Our lab has identified specific types of chromatin that help determine when certain genes are on or off, or that keep a gene poised to be turned on later in development. We leverage emerging technologies in genomics and computation to study chromatin organization across the genome. We use this information to better understand chromatin regulatory processes and how their failure contributes to cancer.

Bernstein Laboratory

Bradley Bernstein, MD, PhD
Sofia Luciana Battaglia
Ryanne Boursiquot
Yotam Drier, PhD
Christine Eyler, MD, PhD
Julie Finn
Will Flavahan, PhD
Liz Gaskell, PhDVolker Hovestadt, PhD
Sarah Johnstone, MD, PhD
Ik Soo Kim, PhD
Kyung Lock Kim, PhD
Yen-Der Li
Fadi Najm, BS
Sid Purham, MD, PhD
Gilbert Rahme, PhD
Sarah Shareef, BS*
Dan Tarjan, BS*
Peter van Galen, PhD
Samanthan J. Vantine
Julia Verga, BS
Jingyi Wu, PhD

* Graduate student

A central question in human biology is how a single genome sequence can give rise to the hundreds of different cell types in the body. Scientists understand that differential patterns of gene expression underlie the many different cellular phenotypes seen in multicellular organisms. However, our understanding of how these gene expression patterns arise during development and how they are subsequently maintained in the adult organism remains poor. A number of studies have indicated that these different expression patterns and phenotypes are intimately related to the way in which genomic DNA is organized into chromatin in the cell. This organizational structure of proteins and DNA, sometimes referred to as the epigenome, helps control which genes are expressed in a given cell type and is critical to the function of normal cells. Moreover, a large body of evidence suggests that the epigenome is inappropriately altered in most—if not all—human cancers.

The long-term goal of our research is to achieve a comprehensive understanding of how the human genome is organized into chromatin. Our group is further focused on understanding how dynamic alterations in chromatin structure contribute to mammalian development and how aberrant chromatin regulation contributes to cancer progression, heterogeneity and therapeutic resistance. We are taking a multifaceted approach involving stem cell biology, biochemistry, genetics, genomics and computational biology. The specific areas of research activity in the lab are explained below.

Technologies for mapping histone modifications and chromatin proteins

We are combining tools in cell biology, biochemistry and molecular biology, with next-generation sequencing to achieve increasingly precise, genome-wide views of chromatin structure, chromatin regulator

The machinery of chromatin regulation

The Bernstein group is focused on understanding the genome-wide regulation and control of chromatin — DNA and its associated proteins. Studies in this group provide views into the 'machinery' that regulates chromatin in mammalian cells, demonstrating that Chromatin Regulators (CRs) act in a similar manner to the way gears function in a machine. In the illustration, the gears represent CRs that may act in concert or alone to control different genomic environments.

Artwork by Lauren Solomon, Alon Goren and Leslie Gaffney, MGH and The Broad Institute. Original photograph from iStockphoto (Maksim Toome, photographer).

binding and genome organization. Integrative analysis of such chromatin state maps yields detailed annotations of the locations and dynamics of functional elements in the human genome, including promoters, transcripts, silencers, insulators and enhancers. Ongoing projects are applying these annotations to understanding cell circuits and how they vary across cell types during development and in cancer.

Epigenetic regulation of stem cell differentiation

Chromatin regulators, such as the Polycomb and trithorax complexes, play critical roles in controlling the expression and potential of genes during development. We identified a novel chromatin structure, termed bivalent domains, that is subject to simultaneous regulation by Polycomb repressors and trithorax activators. Bivalent domains appear to keep developmental regulator genes poised in pluripotent embryonic stem cells and may also serve similar functions in multipotent progenitor cells. Current studies are leveraging a new generation of experimental assays to characterize the functions of bivalent domains and to understand the mechanisms that underlie their establishment and function.

Chromatin regulation in cancer cells

Genes encoding chromatin regulators are frequently mutated in human cancer. In specific cases, these alterations appear to be major drivers of the malignant state. Ongoing studies in the lab seek to apply epigenomic technologies to characterize the transcriptional and epigenetic landscapes of cancer stem cells and to identify mechanisms by which epigenetic changes contribute to therapeutic resistance.

Selected Publications:

Flavahan WA, Drier Y, Liau BB, Gillespie SM, Venteicher AS, Stemmer-Rachamimov AO, Suva ML, **Bernstein BE**. Insulator dysfunction and oncogene activation in IDH mutant gliomas. Nature 2016; 529:110-4.

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Patel AP, Tirosh I, Trombetta JJ, Shalek AK, Gillespie SM, Wakimoto H, Cahill DP, Nahed BV, Curry WT, Martuza RL, Louis DN, Rozenblatt-Rosen O, Suva ML, Regev A, **Bernstein BE**. Single Cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science*. 344:1396-1401, 2014.

Knoechel B, Roderick JE, Williamson KE, Zhu J, Lohr JG, Cotton MJ, Gillespie SM, Fernandez D, Ku M, Wang H, Piccioni F, Silver SJ, Jain M, Pearson D, Kluk MJ, Ott CJ, Shultz LD, Brehm MA, Greiner DL, Gutierrez A, Stegmaier K, Kung AL, Root DE, Bradner JE, Aster JC, Kelliher MA, **Bernstein BE**. An epigenetic mechanism of resistance to targeted therapy in T-cell acute lymphoblastic leukemia. *Nat Genet*. 46: 364-70, 2014.

Suva ML, Riggi N, **Bernstein BE**. Epigenetic reprogramming in cancer. *Science*. 339:1567-70, 2013.

Priscilla K. Brastianos, MD

The Brastianos laboratory studies genomic drivers of human brain tumors. A lack of understanding of the molecular drivers of many brain tumors has hampered the development of novel therapies for many brain cancers. Our overarching objective is to characterize molecular drivers of both progression in primary brain tumors and brain metastases, and accelerate the development of novel therapeutic approaches for these diseases. We recently discovered clinically significant genetic drivers in meningiomas, craniopharyngiomas, hemangioblastomas, glioneuronal tumors and brain metastases. We are currently investigating the role of these genomic drivers as potential therapeutic targets in national NCI-sponsored multi-center clinical trials. Additionally, we are expanding our in vitro and in vivo investigations to further elucidate the molecular evolution of the metastatic process to the central nervous system.

Characterizing Genomic Drivers of Craniopharyngiomas

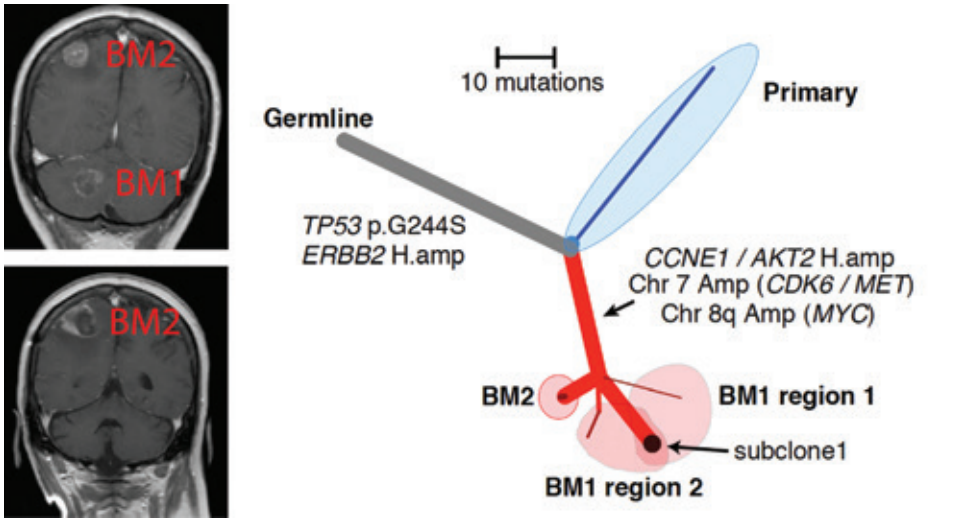
Craniopharyngiomas are epithelial tumors that arise in the pituitary stalk along the path of the craniopharyngeal duct. There are two main subtypes of craniopharyngiomas, the adamantinomatous form that is more common in children, and the papillary form that predominantly occurs in adults. Craniopharyngiomas can cause profound clinical sequelae both through mass effect at presentation and through morbidity of treatment. No effective treatment besides surgery and radiation is known for craniopharyngiomas, and incomplete knowledge of the molecular mechanisms that drive craniopharyngiomas has limited the development of targeted therapies for this tumor. We recently comprehensively characterized the molecular drivers of craniopharyngiomas. We identified activating mutations in CTNNB1 in nearly all adamantinomatous craniopharyngiomas and recurrent mutations in BRAF (resulting in p.Val600Glu) in nearly all papillary craniopharyngiomas. These findings have important implications for the diagnosis and treatment of these neoplasms. We recently treated a patient with multiple recurrent papillary craniopharyngioma with a BRAF and MEK inhibitor and achieved an exceptional therapeutic response. We have initiated a national multicenter trial in craniopharyngiomas to investigate the role of targeted therapies in these tumors. Circulating biomarkers and genomic analysis of craniopharyngiomas will be employed to investigate mechanisms of resistance.

Identifying Molecular Drivers of Meningiomas

Meningiomas are the most common primary nervous system tumor, with no known effective systemic therapy. Recently, we comprehensively characterized meningiomas. Through whole-genome, whole-exome and targeted sequencing, we demonstrated that most meningiomas exhibited simple genomes, with fewer mutations, rearrangements, and copy-number alterations than reported in

Brastianos Laboratory

Christopher Alvarez-Breckenridge, MD
Mia Bertalan
Priscilla K. Brastianos, MD
Ugonma Chukwueke, MD
Taylor Conroy
Husain Danish, MD
Nathaniel Goss
Franziska Marie Ippen, MD
Tareq Juratli, MD
Benjamin Kuter
Matthew Lastrapes
Joana Mora
Naema Nayyar
Brian Shaw
Jackson Stocking
Matthew Strickland, MD
Megha Subramanian, MD, PhD
Michael White, MD
Sally Williams
Michael Young, MD



Representative phylogenetic tree of a primary tumor and 2 anatomically distinct brain metastases. Different regions of the brain metastases shared the same amplifications in CCNE1, AKT2, CDK6, MET and MYC, which were not present in the primary tumor biopsy.

other adult tumors. A subset of meningiomas harbored recurrent oncogenic clinically actionable mutations in AKT1 (E17K) and SMO (W535L). Notably, these mutations were present in therapeutically challenging tumors of the skull base. We also recently identified potential genetics drivers of progression in meningiomas. Because therapeutic targets for SMO and AKT1 mutations are currently in clinical use in other cancers, we are now conducting a prospective national multicenter Phase 2 study of an AKT1, SMO or FAK inhibitors in patients with recurrent or progressive meningiomas harboring AKT1, SMO, or NF2 mutations, respectively. The trial is activated at more than 400 sites throughout the US. We will be genomically characterizing prospectively collected samples to identify biomarkers of response and mechanisms of resistance.

Central Nervous System Metastasis Program

Brain metastases are a common complication of cancer, with a dismal prognosis. There is a limited understanding of the oncogenic alterations harbored by brain metastases and whether these are shared with their primary tumors or other metastatic sites. The objectives of the Central Nervous System Metastasis Program are to (1) identify novel therapeutic targets through comprehensive genomic, transcriptomic and epigenomic characterization, (2) functionally characterize candidate drivers through in vitro and in vivo models of metastasis, and (3) accelerate the application of our scientific findings to the clinical setting. In collaboration with The Broad Institute, and with many national and international institutions, currently we are comprehensively characterizing the genomics of brain metastases to understand the molecular pathways that drive these tumors. Our hope is that the findings from our genomic and functional investigations will allow us to develop more rational therapeutic approaches for this disease.

Selected Publications:

Juratli TA, McCabe D, Nayyar N, ... Carter SL, Cahill DP, **Brastianos PK**. (2018). DMD genomic deletions characterize a subset of progressive/higher-grade meningiomas with poor outcome. *Acta Neuropathologica*. In Press.

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Shankar GM, Abedalthagafi M, Vaubel R, ... Curry WT, Cahill DP*, Barker FG*, **Brastianos PK***, Santagata S*. Germline and somatic BAP1 mutations in high-grade rhabdoid meningiomas. **Neuro-Oncology**. 2017; 19(4):535-545.

Brastianos PK, Shankar GM, Gill CM, Taylor-Weiner A, Nayyar N, Panka DJ, Sullivan RJ, Frederick DT, Abedalthagafi M, Jones PS, Dunn IF, Nahed BV, Romero JM, Louis DN, Getz G, Cahill DP, Santagata S, Curry WT Jr, Barker FG 2nd. Dramatic Response of BRAF V600E Mutant Papillary Craniopharyngioma to Targeted Therapy. *J Natl Cancer Inst*. 2015 Oct 23; 108(2).

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Brastianos PK, Taylor-Weiner A, Manley PE, et al. Exome sequencing identifies BRAF mutations in Papillary craniopharyngiomas. *Nat Genet*. 2014 Feb; 46(2):161-5.

Brastianos PK, Horowitz PM, et al. Genomic sequencing of meningiomas identifies oncogenic SMO and AKT1 mutations. *Nat Genet*. 2013 Mar; 45(3):285-9.

*Co-senior authors

Mark Cobbold, MRCP, PhD

The Cobbold laboratory focuses on understanding how the healthy human immune response is able to recognize and target cancerous cells, and when it fails, how it could be strengthened to recognize this endogenous threat. Our immune system faces a challenge in targeting cancerous cells as they are not "foreign" yet subtle changes in the cellular proteins exist that nevertheless allow our immune cells (T-cells) to detect them. Cancer cells modify internal proteins in different ways to healthy cells, a process fundamental to a cell becoming cancerous. These abnormal modifications can be recognized by T-cells and we believe play a role in protecting us against cancer.

In patients with cancer, this immunity is often lost and therefore new approaches may be needed. We are developing therapeutics that mobilize existing potent immune responses to instead tackle cancer. These immune-based therapies make cancerous cells appear virally infected in the hope that our own anti-viral immunity could step in to fill the void.

Cobbold Laboratory

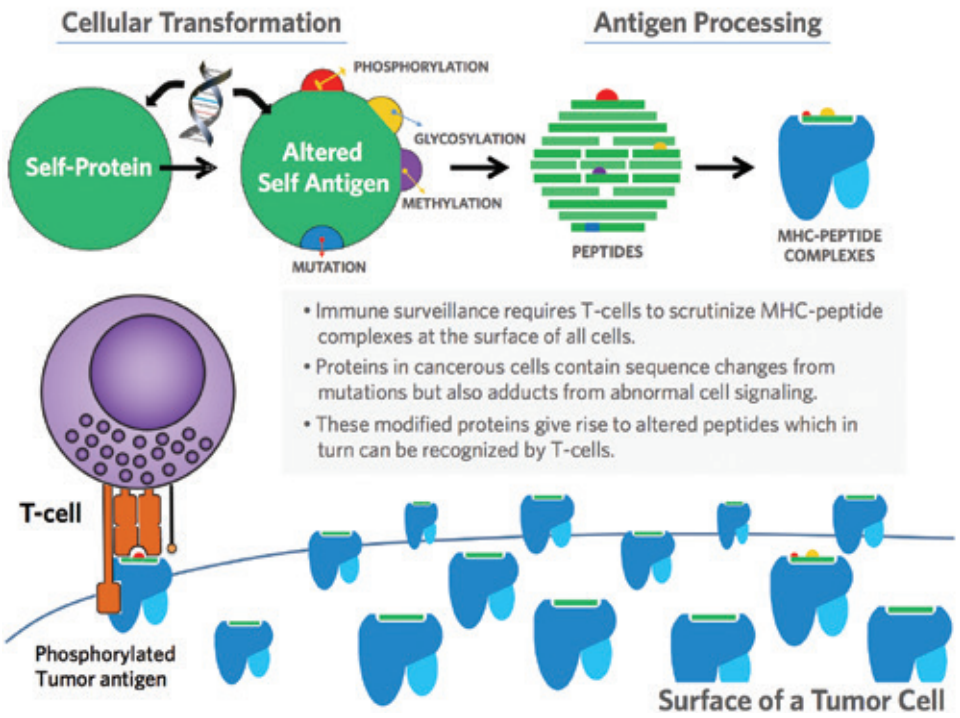
Mark Cobbold, MRCP, PhD
Jamie Heather, PhD
David G. Millar, PhD
Sergio Nunez
Sean Sepulveda
Feng Shi, PhD
Yifang Ivana Shui, PhD
Li Wan,
Songfa Zhang, PhD

Tumor Neoantigens

Recently, new therapies such as checkpoint blockade therapies have revealed potent underlying anti-tumor immunity in patients with many types of cancer. The nature of the tumor antigens targeted by endogenous immunity is still being characterized, but "altered-self" antigens (neoantigens) are thought to play a dominant role. Neoantigens can be generated through genetic mutations that lead to changes in protein sequence (so called "mutational neoantigens"), but these types of antigens are typically unique to each tumor and thus any approach targeting these would need to be in a form of a personalized therapeutic.

We have identified posttranslational modifications (phosphorylation, glycosylation and methylation) as additional mechanisms, whereby proteins can be abnormally modified allowing immune recognition. Thus, aberrant signaling renders phosphate, methyl-, or N-GlcNAc protein adducts as pathophysiological haptens (*Cancer Immunol Res.* 2017). These antigens are abundantly displayed on the surface of cancer cells bound to MHC class-I molecules, and we have identified over 1,000 phosphorylated peptides found on primary tumors. In contrast to classical neoantigens, the surface-display of posttranslationally modified neoantigens is shared between many tumor types potentially affording a broader targeting approach.

This class of tumor antigens exhibits interesting MHC binding with, for example, increased affinity for MHC molecules over non-phosphorylated counterparts. Moreover, structural studies reveal an MHC surface binding pocket facilitating the presentation of the phosphate groups to T-cells (*Nature Immunology* 2008). This recognition pathway may have evolved to allow T-cells to scrutinize underlying cellular signal transduction pathways directly linking adaptive immunity with protection against fundamental oncogenic processes.



Model for generation of modified neoantigens as targets for cancer immunosurveillance.

We have shown the existence of memory-like T-cells in healthy individuals that recognizes leukemia-associated phosphopeptide antigens, yet this immunity is lost in patients with leukemia (*Science Translational Medicine* 2013). Immunotherapies, such as stem cell transplantation where healthy donor immunity is transferred to patients, restore this immunity providing some evidence for its potential utility. Thus posttranslationally modified antigens represent a new class of neoantigens, but their importance in cancer remains to be determined.

T-cell Activating Biotherapeutics

When detected in the blood, immunity against cancer is known to be weak, exhibiting low frequencies and of compromised function. This contrasts starkly against immunity toward persistent viruses such as human cytomegalovirus (CMV) or Epstein-Barr virus (EBV), which typically occupy exceptionally high frequencies in both blood and tissues. Moreover, it is known that CMV immunity increases with age and is preserved in patients with cancer, thus making it ideal to harness against tumors.

We have advanced the notion of altered-self and developed a technology that is able to manipulate the types of antigens present on the surface of tumor cells. We utilized dominant CMV antigens (we term "über-antigens") to develop peptide payloads that could be conjugated to tumor-targeting antibodies. These complexes allow surface release of the über-antigens with subsequent passive loading into empty MHC class-I molecules by exploiting proteases expressed by cancer and engineering antigens that can be processed by these proteases. Thus, this approach circumvents the requirement in the host of potent anti-cancer immunity, which is lacking.

Most recently, we have taken the approach of engineering antibodies that cooperatively engage with immune effector cells to target cancer cells with ultra-specificity through logic gated activation.

Selected Publications:

Wong GK, Heather JM, Barmettler S, **Cobbold M**. Immune dysregulation in immunodeficiency disorders: The role of T-cell receptor sequencing. *J Autoimmun*. 2017 Jun;80:1-9

Malaker SA, Penny SA, Steadman LG, Myers PT, Loke JC, Raghavan M, Bai DL, Shabanowitz J, Hunt DF, **Cobbold M**. Identification of Glycopeptides as Posttranslationally Modified Neoantigens in Leukemia. *Cancer Immunol Res*. 2017 May; 5(5):376-384.

Wong GK, Millar D, Penny S, Heather JM, Mistry P, Buettner N, Bryon J, Huissoon AP & **Cobbold M**. Accelerated Loss of TCR Repertoire Diversity in Common Variable Immunodeficiency. *J. Immunol*. 2016 Sep 1; 197(5):1642-9. PMID: 27481850.

Cobbold M*, De La Peña H, Norris A, Polefrone JM, Qian J, English AM, Cummings KL, Penny S, Turner JE, Cottine J, Abelin JG, Malaker SA, Zarling AL, Huang HW, Goodyear O, Freeman SD, Shabanowitz J, Pratt G, Craddock C, Williams ME, Hunt DF, Engelhard VH. MHC class I-associated phosphopeptides are the targets of memory-like immunity in leukemia. *Sci Transl Med*. 2013 Sep 18; 5(203):203ra125.

Mohammed F†, **Cobbold M**†, Zarling AL, Salim M, Barrett-Wilt GA, Shabanowitz J, Hunt DF, Engelhard VH, Willcox BE. Phosphorylation-dependent interaction between antigenic peptides and MHC class I: a molecular basis for the presentation of transformed self. *Nat Immunol*. 2008 Nov; 9(11):1236-43. Oct 5. PubMed PMID: 18836451.

*Corresponding Author
†Co-first authorship

Ryan Corcoran, MD, PhD

The Corcoran laboratory focuses on developing new and effective therapies for gastrointestinal cancers, including colorectal, pancreatic, stomach, and esophageal cancers, by targeting the specific survival signals that are active in a given patient's cancer. Our research utilizes targeted therapies, which are drugs that inhibit signaling pathways activated by the specific mutations that drive individual tumors. Since cancer cells often become resistant to these targeted therapies by activating alternative signaling pathways, we focus on identifying these key resistance signals in cancer cells. We utilize this information to devise effective combinations of targeted therapies that anticipate and ultimately overcome these mechanisms of drug resistance. Overall, our goal is to develop promising therapeutic strategies that can be evaluated in clinical trials for patients whose cancers are driven by specific mutations.

Corcoran Laboratory

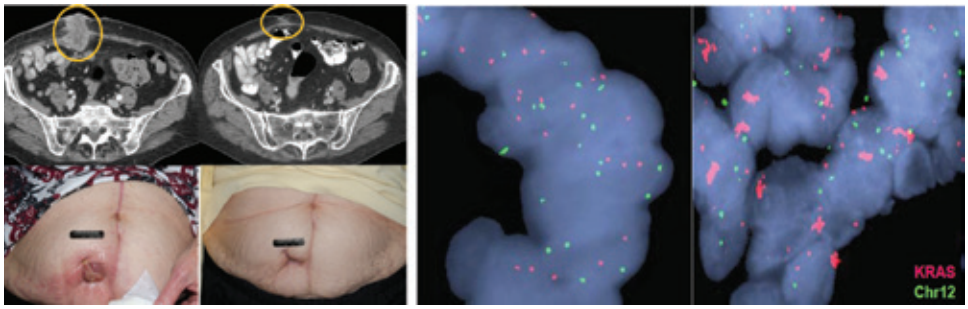
Leanne Ahronian, PhD
William Bradford
Ryan Corcoran, MD, PhD
Ferran Fece De La Cruz, PhD
Isobel Fetter
Ipsita Guha, PhD
Trina Hong
David Myers
Sarah Phat
Meagan Ryan, PhD
Heather Shahzade
Deepinder Singh

Targeted therapy strategies for gastrointestinal cancers

Historically, the standard clinical approach for patients with advanced cancers has been to treat all patients with the same tumor type with the same generalized chemotherapy strategy. However, even among patients with the same type of tumor, the genetic mutations driving tumor growth in each individual patient can be vastly different. As an alternative approach, by identifying the key gene mutations present in an individual patient's tumor, we can "personalize" therapy by matching each patient with specific therapies that target those mutations essential for tumor growth. Our laboratory focuses on developing targeted therapy strategies directed against specific mutations commonly found in gastrointestinal cancers, including cancers with BRAF and KRAS mutations. However, while targeted therapy strategies can lead to dramatic tumor responses, clinical benefit is often limited by the ability of tumor cells to evolve and develop resistance to therapy. By identifying and understanding the key signals driving resistance, our laboratory aims to devise combinations of targeted agents that can overcome or even prevent resistance.

BRAF-mutant colorectal cancer

BRAF mutations occur in 10-15% of colorectal cancers and confer poor prognosis. While BRAF inhibitors have shown dramatic anti-tumor activity in melanomas harboring BRAF mutations, these agents are ineffective in BRAF-mutant colorectal cancers. Therefore, our laboratory has focused on determinants of resistance to BRAF inhibitors in BRAF-mutant colorectal cancers. We have found that reactivation of the MAPK signaling pathway (often mediated through EGFR), contributes to the relative insensitivity of BRAF mutant colorectal cancers to BRAF inhibition. However, we found that combining BRAF inhibitors with EGFR and/or MEK inhibitors can overcome resistance, leading to improved efficacy (*Cancer Discovery,* 2012). We have also identified multiple mechanisms of resistance that can arise to these newer BRAF inhibitor combinations, and are utilizing this information to develop therapeutic strategies to surmount resistance (*Cancer Discovery*, 2015; *Cancer Discovery*, 2018).



Pre-treatment Week 16 Pre-treatment Post-progression

Response and resistance in BRAF-mutant colorectal cancer. (Left) Example of a dramatic tumor response in a patient treated with the combination of a BRAF and a MEK inhibitor. (Right) KRAS amplification (red probes) can lead to BRAF inhibitor resistance in BRAF mutant colorectal cancer patients.

KRAS-mutant cancers

KRAS is the most commonly mutated oncogene in human cancer, mutated in ~20% of all cancers, including pancreatic (~90%) and colorectal cancers (~40%). Currently no effective therapies exist for KRAS-mutant cancers, likely because KRAS itself has proven difficult to target directly with small molecules. Our current work focuses on identifying novel target pathways in KRAS-mutant cancers through hypothesis-based and large-scale pooled RNA interference screening approaches, with the goal of developing new targeted therapy combination approaches for KRAS-mutant cancers. Recently, through a pooled RNA interference drug screen, we identified combined targeting of BCL-XL and MEK as a promising therapeutic strategy that leads to dramatic tumor regressions in KRAS-mutant mouse tumor models. We have also identified adaptive feedback signals that impede the ability of MEK inhibitors to suppress MAPK signaling. We have expanded these approaches to identify other potentially effective targets in KRAS-mutant cancers.

Translational Oncology

The overall goal of our research is to develop improved treatments for patients with gastrointestinal cancers and to identify molecular markers that may help us identify those patients most likely to respond to a given therapy. As such, our laboratory takes a highly translational approach to bringing new therapeutic strategies into the clinic for evaluation in novel clinical trials. Based on our observations, we have launched several clinical trials of BRAF inhibitor combinations in BRAF-mutant colorectal cancers that are showing increased efficacy (*J Clinical Oncology,* 2015). We have also developed a clinical trial combining the BCL-XL/BCL-2 inhibitor navitoclax with the MEK inhibitor trametinib in KRAS-mutant cancers.

To guide our laboratory investigations, we are utilizing key clinical specimens, including tumor biopsies and patient-derived tumor models to understand how tumors become resistant to therapy. We also utilize serial blood collections for circulating tumor DNA analysis to monitor the tumor heterogeneity and clonal dynamics associated with the emergence of therapeutic resistance (*Cancer Discovery* 2015, *Nature Medicine* 2015, *Cancer Discovery* 2016, *Cancer Discovery* 2017, *Cancer Discovery* 2018.)

Selected Publications:

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Goyal L, Saha SK, Liu LY, Siravegna G, Leshchiner I, Ahronian LG, Lennerz JK, Vu P, Desphande V, Kambadakone A, Mussolin B, Reyes S, Henderson L, Sun JE, Van Seventer EE, Gurski JM Jr., Baltschukat S, Schacher-Engstler B, Barys L, Furet P, Ryan DP, Stone JR, Iafrate AJ, Getz G, Porta DG, Tiedt R, Bardelli A, Juric D, **Corcoran RB***, Bardeesy N*, Zhu AX*. Polyclonal secondary FGFR2 mutations drive acquired resistance to FGFR inhibition in FGFR2 fusion-positive cholangiocarcinoma patients. *Cancer Discovery*. 2017; 7: 252-263.

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Kwak EL, Ahronian LG, Siravegna G, Mussolin B, Godfrey JT, Clark JW, Blaszkowsky LS, Ryan DP, Lennerz JK, Iafrate AJ, Bardelli A, Hong TS, **Corcoran RB**. Molecular heterogeneity and receptor co-amplification drive resistance to targeted therapy in MET-amplified esophagogastric cancer. *Cancer Discovery*. 2015; 5: 1271-81.

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*Denotes equal contribution

Shawn Demehri, MD, PhD

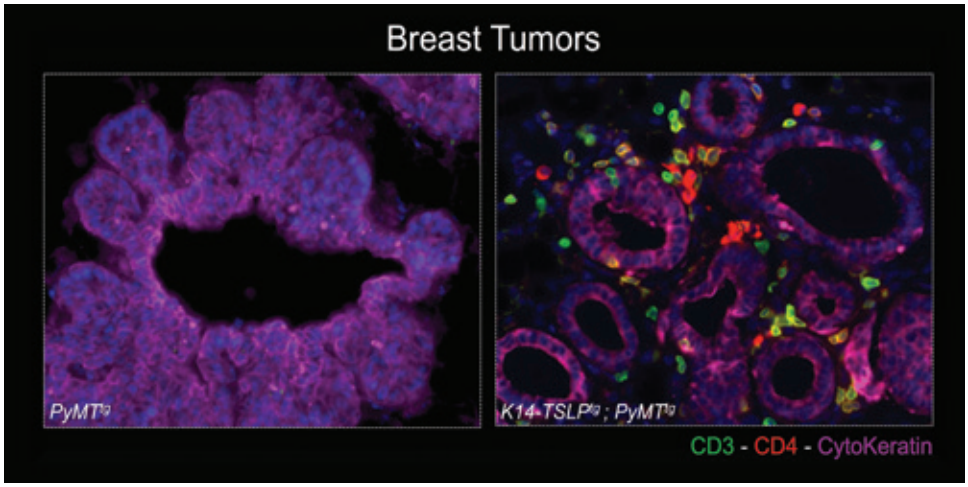
The focus of **the Demehri laboratory** is to determine the role of the immune system in regulating the early stages of cancer development in order to harness its anti-tumor potential for cancer prevention and treatment. To date, several cancer immunotherapies have been developed with proven efficacy against late-stage cancers; however, the role of the immune system in preventing the early development of cancer remains uncertain. The research in our laboratory is focused on identifying the immune mechanisms that drive an immune activation sufficient to prevent cancer formation from pre-cancerous lesions. This approach raises a great opportunity to discover novel immune pathways that can be leveraged in cancer therapy and prevention.

Demehri Laboratory

Amir Ameri
Mary Awad
Margherita Boieri, PhD
Mark Bunting, PhD
Shawn Demehri, MD, PhD
Kaitlin Dempsey
Tatsuya Hasegawa, PhD
Isabella Kopits
Kaiwen Li, MD
Tiancheng Li, MD
Elena Lopez
Johnathan Messerschmidt
Kenneth Ngo
Jongho Park, PhD
Erik Schiferle

The field of cancer immunology has made substantial advances in recent years by deciphering the role of the tumor infiltrating CD8+ cytotoxic T lymphocytes (CTLs) in attacking cancer cells, which have led to promising new cancer immunotherapeutics. The current immunotherapeutic approaches, however, are largely designed to boost the anti-tumor immune response that has already formed against late-stage metastatic cancers. Therefore, the current cancer immunotherapies like immune checkpoint blockade, which rely on a pre-existing CTL infiltrate in the tumor for their effects, are proven ineffective to treat cancers that frequently lack a significant anti-tumor immune infiltrate, especially during the early in-situ phases of their development. In order to expand the potential of cancer immunotherapy, our laboratory studies the pathways that lead to immune system activation against early phases of cancer development. Devising a mechanism to activate the immune system against early-stage cancers has clear immunopreventive implications by directly blocking the cancer promotion and immunotherapeutic benefits by potentiating the immunity against late disease.

To pursue this goal, our laboratory is currently focused on three areas of research: 1) Mechanisms of CD4+ T cell activation against cancer. Our laboratory has studied the mechanism of thymic stromal lymphopoietin (TSLP) in evoking tumor suppression. TSLP is an epithelial-derived cytokine that plays a central role in stimulating CD4+ T helper 2 (Th2)-mediated allergic diseases like atopic dermatitis and asthma. We have shown that high TSLP levels establish a dominant anti-tumorigenic immune environment preventing cancer promotion. Currently, our team investigates the detailed mechanism of TSLP anti-tumor function against solid cancers and examines its application for the treatment of pre-cancerous skin and breast lesions in patients. 2) Mechanisms of natural killer (NK) cell recruitment and activation against cancer. NK cells are known for their potent anti-tumor properties. However, their role in controlling the cancer development in vivo remains unclear. Our laboratory is utilizing a virally encoded ligand for NK cells to determine the combination of signals necessary to activate NK cells against early stages of carcinogenesis and to identify the mechanism of anti-tumor immunity mounted by the activated NK



CD4+ T cell immunity against breast cancer development.

cells in order to block cancer promotion and progression. 3) Mechanisms of tumor promotion by the immune system. Although immune cells can mount anti-tumor immunity against cancer, they are also implicated in promoting cancer development under certain conditions. Chronic inflammation is one of the conditions that can predispose patients to cancer; however, the mechanism of such immune-mediated tumor promotion is unclear. To determine this mechanism, our laboratory studies skin and colorectal cancer development as ideal cancer models in which the spatial and temporal relationship between inflammation and cancer development can be determined with exceptional precision. We are currently investigating the immune mechanisms that promote skin cancer development in the context of chronic allergic contact dermatitis and cutaneous lupus and colorectal cancer development in the context inflammatory bowel disease.

Selected Publications:

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Demehri S, Cunningham TJ, Manivasagam S, Ngo KH, Moradi Tuchayi S, Reddy R, Meyers MA, DeNardo DG, and Yokoyama WM. Thymic stromal lymphopoietin blocks early stages of breast carcinogenesis. *J Clin Invest*. 2016; 126:1458-1470.

Demehri S, Turkoz A, Manivasagam S, Yockey LJ, Turkoz M, and Kopan R. Elevated epidermal thymic stromal lymphopoietin levels establish an antitumor environment in the skin. *Cancer cell*. 2012; 22, 494-505.

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Nicholas Dyson, PhD

The Dyson laboratory studies the role of the retinoblastoma tumor suppressor (RB). RB is expressed in most cell types and its functions enable cells to stop dividing. RB is inactivated in many types of cancer. We have three main goals: we want to understand the molecular details of how RB acts, we want to know how the inactivation of RB changes the cell, and we are using these insights to target tumor cells.

Dyson Laboratory

Brian Brannigan
Benjamin Drapkin, MD, PhD
Nicholas Dyson, PhD
Ana Peralta Guarner, PhD
Badri Krishnan, PhD
Sarah Phat, MS
Purva Rumde, BS
Ioannis Sanidas, PhD
Marcello Stanzione, PhD
Jun Zhong, BS

My laboratory investigates mechanisms that limit cell proliferation in normal cells and the ways that these controls are eroded in cancer cells. Our research focuses on RB, the protein product of the retinoblastoma susceptibility gene (*RB1*), and on E2F, a transcription factor regulated by RB. RB/E2F control the expression of a large number of genes that are needed for cell proliferation. This transcription program is activated when normal cells are instructed to divide but it is deregulated in tumor cells, providing a cellular environment that is permissive for uncontrolled proliferation. RB has multiple activities but one of its key roles is to limit the transcription of E2F targets. As a result, most tumor cells select for changes that compromise RB function. Our research program spans three areas of RB/E2F biology.

Dissecting the molecular functions of RB

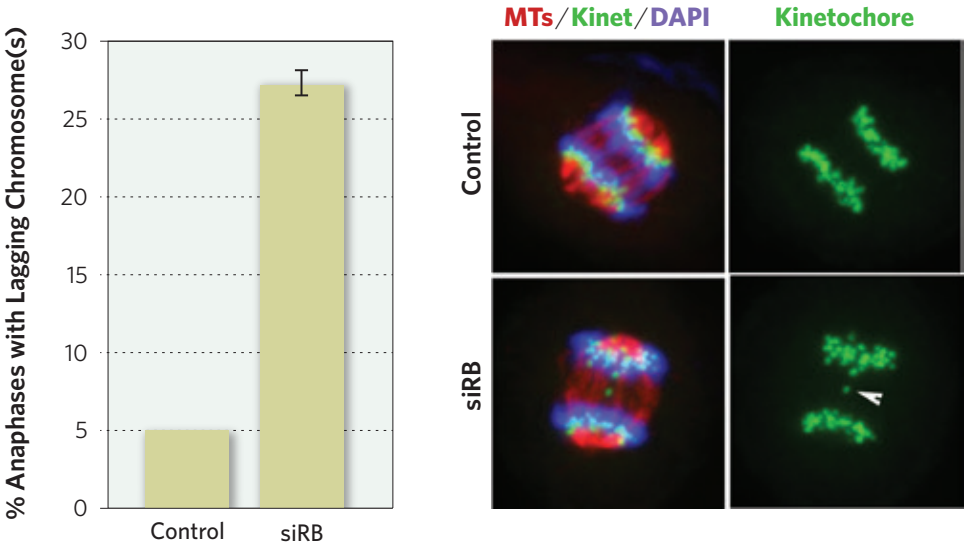
RB's precise mechanism of action remains an enigma. RB has been linked to hundreds of proteins and has been implicated in many cellular processes. However, purification of endogenous RB complexes has been a major challenge and, consequently, it is uncertain which proteins physically interact with RB in any specific context. We have solved this problem and, in collaboration with the Haas lab, are using Mass Spectrometry to take detailed snapshots of RB in action. We have used these technologies to examine the hypothesis that RB's activity is tailored by mono-phosphorylation. Our data shows that the various mono-phosphorylated forms of RB interact with different cellular proteins, regulate different sets of genes and have distinct functional properties.

Proteomic profiles give a new perspective on the effects of RB loss

We have also used proteomic and metabolic profiling to better understand the impact of RB loss on a cell. Since RB and E2F are both transcription factors, the effects of RB loss are typically studied by examining changes in the levels of mRNAs synthesized from target genes. Our results show that this RNA-centric approach gives an incomplete picture of the changes that occur following RB loss. Although changes in cell cycle-regulated genes are thought to be hallmarks of RB inactivation, the proteomic changes associated with RB loss are dominated by changes in mitochondrial proteins and the metabolic profiles show changes in glycolysis and in purine and pyrimidine synthesis. These changes may be useful biomarkers in tumor samples, but they may also highlight vulnerabilities of RB1-mutant tumor cells.

Targeting tumor cells with RB1 mutations

Our long-term goal is to use information gleaned from these molecular studies to



Although RB is known to act during G1, the depletion of RB results in a high frequency of lagging chromosomes during Mitosis. RB was selectively depleted from RPE1 cells, a non-transformed cell line, and the appearance of lagging chromosomes during anaphase was scored in cells stained to show the kinetochore (green), microtubules (red) or DNA (blue). Refer to Manning AL et al, Genes Dev. 2010; 24(13):1364-76 for details.

improve cancer treatment. RB is functionally compromised in most types of cancer, but the specific mutation of the *RB1* gene is a hallmark of just three tumor types (retinoblastoma, osteosarcoma and small cell lung cancer (SCLC)). This implies that the complete elimination of RB function is especially important in these tumors. In collaboration with Dr. Anna Farago, our clinical collaborator, and with help from members of the Haber/Maheswaran laboratories we have generated an extensive panel of patient derived xenograft (PDX) models of SCLC. These PDX models accurately reflect the genomic features and the drug sensitivities of the tumors from which they were derived. We are now using this panel of models to compare the effectiveness of different therapies, and to understand which SCLC tumors respond best to each type of treatment. Our goal is to use these models to identify new strategies for targeting RB1-mutant tumors.

Selected Publications:

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Dyson NJ. RB1: a prototype tumor suppressor and an enigma. *Genes and Development*. 2016; 30(13):1492-502.

Nicolay BN, Danielian PS, Kottakis F, Lapek JD, Sanidas I, Miles WO, Dehnad M, Tschop K, Gierut J, Manning AL, Morris R, Haigis K, Bardeesy N, Lees JA, Haas W, and **Dyson NJ**. Proteomic analysis of pRb loss highlights a signature of decreased mitochondrial oxidative phosphorylation. *Genes and Development*. 2015; 29(17):1875-89.

Andrew Elia, MD, PhD

In response to DNA damage from environmental or endogenous sources, cells evoke an elaborate signaling network known as the DNA damage response (DDR). This response functions to preserve genomic integrity, which is necessary for normal development and the prevention of cancer. **The Elia laboratory** studies the DNA damage response, with current projects focusing on DDR pathways regulated by ubiquitin-dependent signaling and DDR pathways that promote the stabilization and repair of stalled replication forks. We utilize innovative proteomic and genetic approaches to investigate these processes. Our ultimate goal is to understand how DDR disruption influences cancer progression and can be exploited to target tumors with specific DNA repair defects.

• • •

Elia Laboratory

Alec Boardman, MD
Andrew Elia, MD, PhD
Ece Kocak, BS
Chandler Moore, BS
Benjamin Wardwell
David Wang, BS

DNA damage response

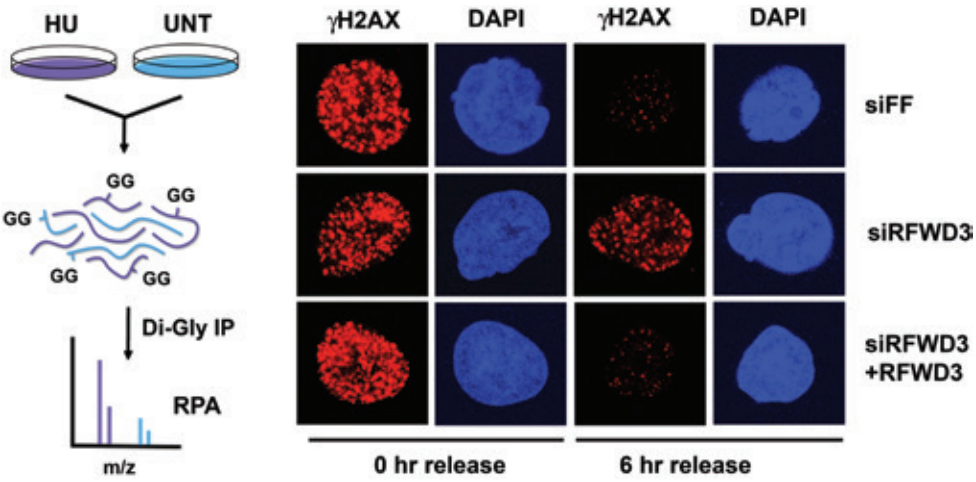
DNA within cells is under continual assault from metabolic and environmental sources. In response to the ensuing damage, cells activate a signaling network called the DNA damage response (DDR). Defects in this response are responsible for numerous hereditary cancer syndromes and can underlie the genomic instability which is a hallmark of many sporadic cancers. The DDR promotes genomic integrity by targeting hundreds of factors in diverse pathways ranging from DNA replication and repair to cell-cycle arrest, senescence, and immune regulation. While much is known about these core pathways, the complex regulatory events coordinating them are less well understood. Our lab aims to elucidate biochemical and genetic relationships between DDR factors to understand how they are integrated and collectively regulated.

Quantitative proteomics in ubiquitin signaling

Execution of the DDR relies upon a dynamic array of protein modifications, with phosphorylation playing a historically central role. It is now evident that the DDR also depends on ubiquitin signaling. Numerous ubiquitin ligases have been implicated in the response, yet finding their substrates by simple binding techniques can be difficult due to weak substrate interactions. To circumvent this problem, we have pioneered a quantitative proteomic approach to globally profile ubiquitination. Initially, we used this approach to identify substrates of Cullin-RING ubiquitin ligases (*Cell* 2011), which are involved in numerous DNA repair processes. Subsequently, we used it to uncover novel ubiquitination events directly stimulated by DNA damage (*Mol Cell* 2015a), demonstrating the vast breadth of ubiquitin signaling in the DDR. We are continuing to use innovative proteomic approaches to characterize novel and poorly understood ubiquitin ligases in DNA damage signaling pathways.

Replication stress and cancer

Replication fork collapse can induce chromosome instability and mutagenic events that cause cancer. Organisms have therefore evolved pathways to stabilize stalled replication forks and to repair collapsed



(Left) Quantitative proteomics identifies RPA ubiquitinaton mediated by the ubiquitin ligase RFWD3, which is mutated in the cancer predisposition syndrome Fanconi anemia. (Right) Depletion of RFWD3 inhibits the repair of collapsed replication forks, as demonstrated by delayed resolution of γH2AX foci six hours after release from hydroxyurea-induced replication fork stalling and collapse.

forks through processes such as homologous recombination (HR). Multiple factors involved in HR and replication fork stabilization, such as BRCA1 and BRCA2, are mutated in hereditary cancer syndromes, highlighting the importance of these pathways. We have demonstrated that the ubiquitin ligase RFWD3, which is mutated in the cancer predisposition syndrome Fanconi anemia, ubiquitinates the single-stranded DNA binding protein RPA to promote homologous recombination at stalled replication forks and replication fork restart (Mol Cell 2015b). We are currently studying RFWD3 function in the replication stress response and elucidating novel mechanisms of replication fork stabilization and repair.

Targeted cancer therapy

Defects in the DNA damage response can render tumors dependent upon specific DNA repair pathways for survival. Moreover, targeted modulation of the DDR can affect tumor sensitivity to genotoxic chemotherapy and radiation. Increased understanding of DNA repair pathways will lead to enhanced opportunities for developing therapies that target cancers with DNA repair defects, and for improving the efficacy of genotoxic treatments. We are employing methods to translate our work to the development of such therapies.

Selected Publications:

Zhou C*, **Elia AE***, Naylor ML, Dephoure N, Ballif BA, Goel G, Xu Q, Ng A, Chou DM, Xavier RJ, Gygi SP, Elledge SJ. Profiling DNA damage-induced phosphorylation in budding yeast reveals diverse signaling networks. *Proc Natl Acad Sci U S A.* 2016; 113(26):E3667-75

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Emanuele MJ, **Elia AE**, Xu Q, Thoma CR, Izhar L, Leng Y, Guo A, Chen YN, Rush J, Hsu PW, Yen HC, Elledge SJ. Global identification of modular cullin-RING ligase substrates. *Cell.* 2011; 147(2):459-74.

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Elia AE, Rellos P, Haire LF, Chao JW, Ivins FJ, Hoepker K, Mohammad D, Cantley LC, Smerdon SJ, Yaffe MB. The molecular basis for phosphodependent substrate targeting and regulation of Plks by the Polo-box domain. *Cell.* 2003; 115:83-95.

*Co-first authors

Leif William Ellisen, MD, PhD

Cancer therapy is being revolutionized through the development of more specific and less toxic treatment approaches that are collectively known as targeted therapeutics. A key to the successful application of targeted cancer therapy is the identification of specific genetic abnormalities within tumor cells that are not present in normal tissues. **The Ellisen laboratory** is broadly interested in identifying these genetic abnormalities, understanding how they influence the biology of cancer cells, and discovering how that biology can inform the selection of the most effective therapy for each patient. We address these questions through basic research studies of key tumor-cell signaling pathways, and through molecular analysis of patient tumor samples conducted in partnership with collaborators in the fields of molecular diagnostics and computational biology. Our discoveries in the basic laboratory and through tumor analysis have already been translated to clinical trials that seek to identify new predictive markers, and new therapeutic strategies for breast and other cancers.

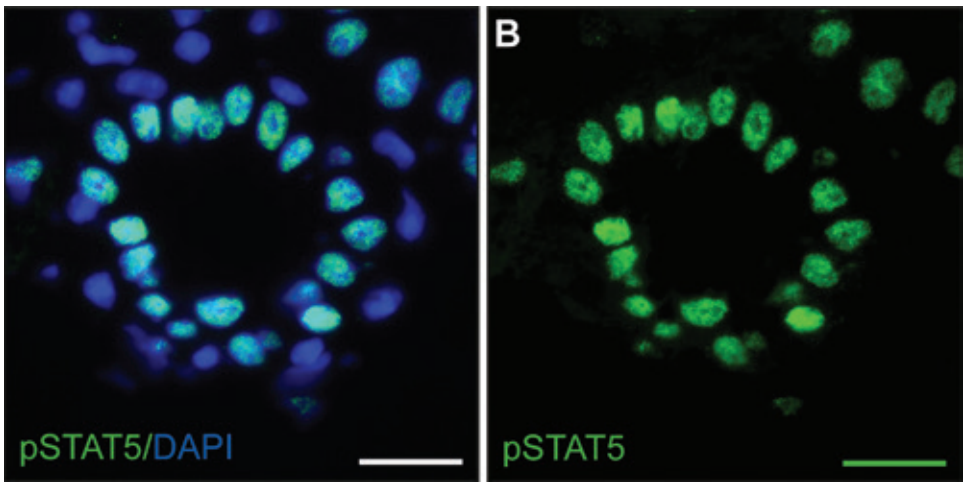
Ellisen Laboratory

Ning Ding, PhD
Leif William Ellisen, MD, PhD
Mihriban Karaayvaz, PhD
Siang Boon Koh, PhD
Aiko Nagayama, MD
Shuxi Qiao, PhD
Srinivas Vinod Saladi, PhD
Sheng Sun, PhD
Varunika Vivekanandan, BS

Our group is broadly interested in how genetic abnormalities of breast and related cancers influence tumor biology, and how that biology can, in turn, be exploited to therapeutic advantage. We address these questions through basic research studies of key tumor cell signaling pathways including p53, mTOR, and BRCA1/2. This work is complemented by molecular analysis of patient tumor samples conducted in partnership with collaborators in the fields of molecular diagnostics and computational biology. Our discoveries in the basic laboratory and through human tumor analysis are being applied in ongoing clinical trials that seek to identify predictive markers of response to specific therapeutics for breast and other cancers. Our ability to work at the interface of basic tumor biology and therapeutic application is strongly supported by our network of collaborators and by the research and clinical infrastructure of the Mass General Cancer Center.

The p53 network in cancer biology and therapy

The p53 tumor suppressor is inactivated in more than 50% of sporadic human cancers, and patients carrying heterozygous germline p53 mutations show striking tumor predisposition. As a transcription factor and key nodal point for integrating cellular responses to DNA damage, p53 regulates genes involved in diverse cellular processes including cell cycle progression, apoptosis and angiogenesis. Through analysis of two p53-related genes, p63 and p73, we and others have defined a functional network through which these factors interact in human tumorigenesis. We have further defined a tissue-specific role for p63 as the enforcer of an epigenetically-controlled progenitor state. These findings are likely to explain the observation that p63 is over-expressed in a broad variety of epithelial tumors, particularly squamous cell and breast carcinomas. Our success in defining such



The lactating mammary alveolus (shown) requires activation of STAT5 (pSTAT5, green/aqua) in luminal cells, which is controlled by paracrine hormonal signaling from basal cells (blue). Loss of this signaling may block luminal differentiation and predispose to breast cancer.

novel functional interactions and contributions for the p53 family provides new therapeutic possibilities for multiple treatment-refractory malignancies.

P53 and TOR-associated metabolic reprogramming in tumorigenesis

Our efforts to identify new pathways regulated by p53 family members have yielded surprising insights into the re-wiring of cellular metabolism that drives carcinogenesis. A central player in this effect is REDD1, a p53-regulated gene we identified that functions as a critical regulator of redox status and the mechanistic Target of Rapamycin (mTOR) kinase. Most human tumors exhibit abnormalities of p53 and/or mTOR signaling, and our recent studies have demonstrated the contribution of REDD1 to autophagy and metabolic homeostasis during tumorigenesis. We are currently using animal models, in vitro studies, and biochemical approaches to understand key metabolic dependencies of tumors that can be exploited to therapeutic advantage.

BRCA1/2, hereditary cancer predisposition and triple-negative breast cancer

Germline mutations in the DNA repair genes BRCA1 and BRCA2 confer dramatically elevated risk of cancers of the breast, ovary, and pancreas, yet the precise pathogenesis of BRCA1/2-associated cancer remains to be elucidated. We have launched a systematic study of early events that give rise to these cancers, in part through detailed molecular analysis of normal and pre-cancerous tissues from BRCA1/2 mutation carriers. Defining the altered signaling and early cooperating events in this context is likely to reveal new markers of breast cancer predisposition and new targets for prevention. This work is complemented by our detailed studies of triple-negative breast cancer (TNBC), an aggressive subtype that comprises 80% of tumors in BRCA1 mutation carriers. Our newly-launched Triple-Negative Breast Cancer Program integrates basic research, translational and clinical studies together with human tumor propagation and high-throughput drug screening, all focused on overcoming drug resistance and improving outcomes for patients with TNBC.

Selected Publications:

Karaayvaz M, Cristea S, Gillespie SM, Patel AP, Mylvaganam R, Luo CC, Specht MC, Bernstein BE, Michor F, and **Ellisen LW**. Unravelling subclonal heterogeneity and aggressive disease states in TNBC through single-cell RNA-seq. *Nature Communications.* 2018 9:3588-97.

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David E. Fisher, MD, PhD

The Fisher laboratory focuses on mechanistic studies which underlie the biology and pathophysiology of skin and melanoma. Research studies range from molecular analyses of pigment cell biology to risk factors responsible for the formation of melanoma and other skin cancers. The laboratory utilizes deep molecular tools to understand how genes are regulated, how they contribute to cancer formation, and how they may be successfully targeted by drugs in order to improve disease treatments or to prevent disease formation altogether. Several areas of particular focus include 1) the study of redhair, fair skinned pigmentation and the manner in which such individuals are at increased risk for skin cancer; 2) identification and analysis of oncogenes which control melanoma cell survival; 3) discovery of new drugs that affect pigmentation, melanoma survival, and other skin-related effects; and 4) examination of the ways in which a gene called MITF plays a master-regulatory role in specifying the development of pigment-producing cells in the body.

Fisher Laboratory

Jennifer Allouche, PhD
Elizabeth Byrne**
Yeon Sook Choi, PhD
Tal Erlich, PhD
Yang Feng*
David E. Fisher, MD, PhD
Laura Gee
Sharon K. Germana
Andrea Hermann
Jennifer Hsiao*
Shinichiro Kato, PhD
Akinori Kawakami, MD, PhD
Lajos Kemeny†
Nhu Nguyen*
Stephen Ostrowski, MD, PhD
Inbal Rahamin, PhD
James Sefton
Mack Su**
Xunwei Wu, PhD
Yao Zhan, PhD

* PhD candidate
** MD-PhD candidate
†Medical Student

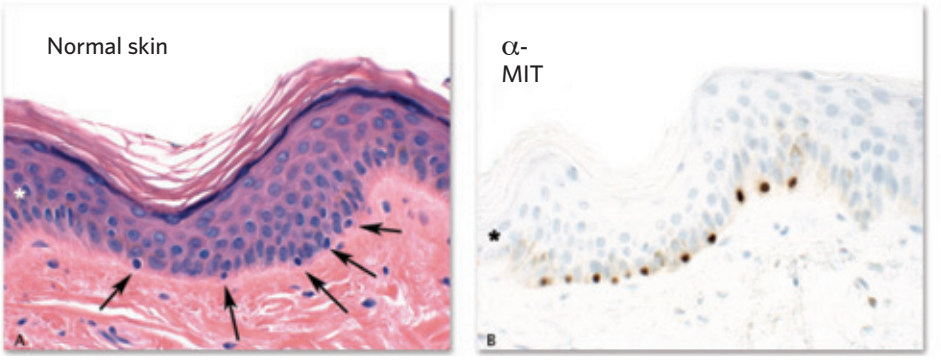
Our group studies cell death/proliferation signals in relation to development and disease, particularly in cancer of pigment cells (melanoma) and tumors of childhood. We attempt to understand critical modes of cell homeostasis with a goal of molecular targeted therapy as well as prevention of melanoma and other human cancers. Areas of particular focus are explained below.

Lessons for malignancy from normal development

We study the biology of melanocytes as a means of identifying pathways which drive human melanoma. This area of research includes examination of the mechanisms underlying the growth/survival of benign moles, most of which contain mutations in either BRAF or N-Ras oncogenes. We also study melanocyte death in hair follicles, a process associated with hair graying. Our work led to the identification of pathways linking graying to melanocyte and melanoma survival, offering potential leads for novel therapies. Other studies focus on pathways modulating melanocytic responses to environmental cues and employ oncogene-transformed melanocytic lines which exhibit growth factor independence, mimicking human melanoma in a genetically controlled manner, and clinical analyses of novel melanoma treatments. We also study the role of UV in pigmentation responses and carcinogenesis.

Control of life and death in melanoma

Malignant transformation of melanocytes produces one of the most treatment-resistant malignancies in human cancers. We have identified a transcriptional network that regulates melanoma cell survival and proliferation and melanocyte differentiation during development. Using diverse methods—including mouse models, human tumor expression arrays, and cellular assays— we



Histologic images of human skin. Left image shows hematoxylin and eosin (H&E) stain. The top layer is Stratum Corneum (consisting of dead cell derivatives) followed by the deeper purple keratinocyte cell layers constituting the epidermis. Beneath the epidermis is the pink, collagen containing dermis. Melanocytes reside at the base of the epidermis and are highlighted by arrows. The image to the right shows antibody staining for the melanocytic transcription factor MITF, which highlights the melanocytes at the dermal-epidermal junction. Histologic images were generated by Dr. Scott Granter.

examine mechanisms through which melanoma cells evade death with the goal of improving therapy. Studies include preclinical and clinical analyses of novel melanoma treatments. We also study the role of UV in pigmentation responses and carcinogenesis.

MITF transcription factor family in development and cancer

MITF is a helix-loop-helix factor homologous to the Myc gene which, when mutated in humans, produces absence of melanocytes. MITF acts as a master regulator of melanocyte development and is targeted by several critical signaling pathways. Recently, members of the MITF family have been identified as oncogenes in a variety of human malignancies, particularly sarcomas of childhood. We are currently investigating their roles in cancer as well as strategies to target them therapeutically. Detailed mechanistic studies focus on transcription factor interactions with chromatin, and epigenetic control of gene expression.

Selected Publications:

Du W, Seah I, Bouqazzoul O, Choi G, Meeth K, Bosenberg MW, Wakimoto H, **Fisher DE**, Shah K. Stem cell-released oncolytic herpes simplex virus has therapeutic efficacy in brain metastatic melanomas. *Proc Natl Acad of Sciences USA* 2017 July 25.

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Friedman A, Xia Y, Trippa L, Phi Le L, Igras V, Frederick D, Wargo JA, Tanabe KK, Lawrence DP, Neuberg DS, Flaherty KT, **Fisher DE**. Feasibility of ultra-high-throughput functional screening of melanoma biopsies for discovery of novel cancer drug combinations. *Clin Cancer Res*. 2017 Apr 26.

Herzberg B, **Fisher DE**. Metastatic melanoma and immunotherapy. *Clin Immunol*. 2016 Nov;172:105-110. PMID: 27430520.

Lin WM, **Fisher DE**. Signaling and Immune Regulation in Melanoma Development and Responses to Therapy. *Annu Rev Pathol*. 2017 Jan 24;12:75-102. PMID: 27959628.

Wang H, Osseiran S, Igras V, Nichols AJ, Roider EM, Pruessner J, Tsao H, **Fisher DE**, Evans CL. In vivo Coherent Raman Imaging of the Melanomagenesis-associated Pigment Pheomelanin. *Scientific Reports*. 6: Article number 37986; 2016. PMCID: PMC5125099.

Gad Getz, PhD

The Getz laboratory is focused on cancer genome analysis, which includes two major steps (i) *Characterization* – cataloging of all genomic events and the mechanisms that created them during the clonal evolution of the cancer, including events at the DNA, RNA and protein levels in normal and tumor samples from an individual patient; and (ii) *Interpretation – analysis* of the characterization data across a cohort of patients with the aim of identifying the alterations in genes and pathways that cause cancer or increase its risk as well as identifying mutational processes, tumor evolution and heterogeneity, molecular subtypes of the disease, their markers and relationship to clinical variables. In addition to developing tools for high throughput analysis of cancer data and experimentally testing the findings, the Getz lab develops compute platforms that enable large-scale analytics and visualization, such as FireCloud, TumorPortal and others.

Getz Laboratory

François Aguet, PhD
Eila Arich-Landkof
Chet Birger, PhD
Liudmila Elagina
Samuel Freeman
Gad Getz, PhD
Aaron Graubert
Megan Hanna
Nicholas Haradhvala
David Heiman
Julian Hess
Vicky Horst
Eliza Katz
Jaegil Kim, PhD
Kirsten Kübler, MD, PhD
Ignaty Leshchiner, PhD
Liza Leshchiner, PhD
Xiao Li
Ziao Lin
Dimitri Livitz
Yosef Maruvka, PhD
Sam Meier
Michael Noble
Paz Polak, PhD
Esther Rheinbay, PhD
Daniel Rosebrock
Gordon Saksena
Eddie Salinas
Ayellet Segrè, PhD
Kara Slowik
Chip Stewart, PhD
Amaro Taylor-Weiner
Keren Yizhak, PhD
Hailei Zhang, PhD

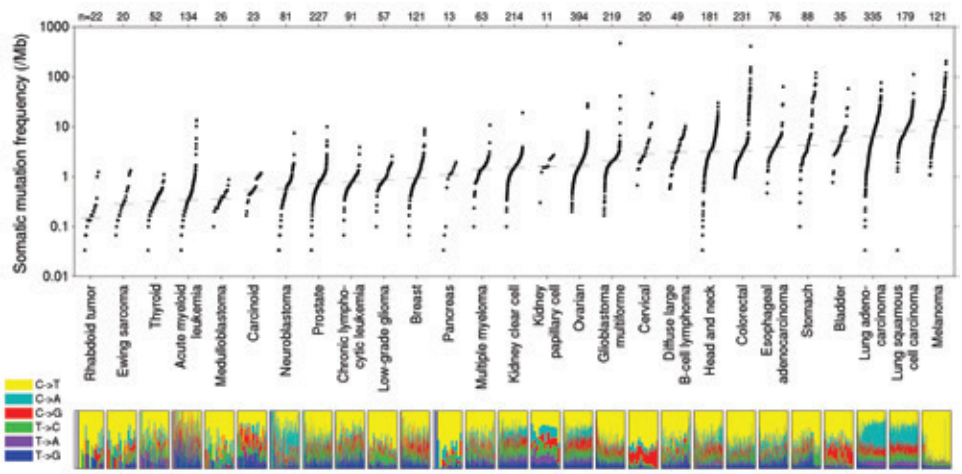
Characterizing the Cancer Genome

Cancer is a disease of the genome that is driven by a combination of possible germline risk-alleles, together with a few 'driver' somatic mutations that increase fitness and promote clonal expansion. Mutations occur at all levels and scales, including DNA point mutations, small insertions and deletions, larger genomic rearrangements and copy-number alterations, as well as epigenetic, transcriptional and proteomic changes. To generate a comprehensive list of all germline and somatic events that occurred during and prior to development of the cancer, we are developing and applying highly sensitive and specific tools for detecting these events in sequencing data. The complexity of the underlying cancer genomes requires the use of state-of-the-art statistical and machine learning approaches to most efficiently extract the signal from the noise.

Detecting Cancer-Associated Genes

Once we detect the events in the cancer genomes, we analyze them across a cohort of samples searching for genes (and pathways) that show significant signals of positive selection, e.g. the number of mutations exceeds what is expected by chance. To do so, we construct a detailed statistical model of the background mutational processes and detect genes that deviate from it. We have developed tools for discovering significantly gained or lost genes in cancer (GISTIC) and genes with increased density or irregular patterns of mutations (MutSig, CLUMPS). In these analyses, it is critical to correctly model the heterogeneity of mutational processes across patients, sequence contexts and the genome. We are constantly improving methods and working towards a unified method for types of alterations.

We recently studied mutations in non-coding regions of the genome in breast cancer and identified several driver promoter mutations, including hotspot mutations in the promoter of *FOXA1*. We further show that these likely increase expression of *FOXA1* by creating E2F binding sites. The lab is continuing to study non-coding drivers as part of a large international effort.



Somatic mutation frequencies across cancer.
Each dot represents the total frequency of somatic mutations (in the exome) in each tumor–normal pair. Tumor types are ordered by their median somatic mutation frequency, from haematological and paediatric tumors (left), to tumours induced by carcinogens such as tobacco smoke and ultraviolet light (right). Mutation frequencies vary more than 1,000-fold between lowest and highest across different cancers and also within several tumour types. The bottom panel shows the relative proportions of the six different possible base-pair substitutions. Taken from Lawrence et al. (2013).

Heterogeneity and Clonal Evolution of Cancer

Cancer samples are heterogeneous, containing a mixture of normal (i.e. non-cancer) cells and a population of cancer cells that often represents multiple subclones. Since cancer is a dynamic system, these subclones may represent the remaining cells of less-fit clones that have not yet been overtaken by the expanding most-fit clone or they may represent interacting sub-clones that co-evolved to support each other and reached an equilibrium or a combination of these scenarios. Our lab has been developing tools (ABSOLUTE, Phylogic) for characterizing the heterogeneity of cancer samples using copy-number, mutational and other data measured on bulk samples and single cells. Using these tools, we can infer which mutations are clonal (i.e. exist in all cancer cells) or sub-clonal (i.e. exist in subclones), as well as estimate the number of subclones and monitor their evolution over time or space by studying multiple samples from the same patient. Recently, we demonstrated that sub-clonal driver mutations are associated with outcome, emphasizing the importance of including clonal information in clinical trials.

Mutational Processes

Mutations are the product of multiple processes that damage, repair, replicate and deliberately alter DNA. We use mutation data to study these processes, understand their mutational signatures, infer their molecular mechanisms and identify alterations that are associated with their activity. We found an association between mutations in *ERCC2* and a specific mutational signature. By studying asymmetries in mutational processes we were able to detect that a mechanism that works on the lagging strand of DNA while it is replicated and a new mutational process that generates mutations on the non-transcribed strand. In a recent study, we use the association between a mutational signature and homologous recombination (HR) defects to show that epigenetic silencing of *RAD51C*, a member of the HR pathway, is an important mechanism for HR deficiency in breast cancer. Moreover, we show that the signature, together with loss-of-heterozygosity can identify germline functional alleles in *BRCA1/2*.

Selected Publications:

Polak P, Kim J, Braunstein LZ, Karlic R, Haradhavala NJ, Tiao G, Rosebrock D, Livitz D, Kübler K, Mouw KW, Kamburov A, Maruvka YE, Leshchiner I, Lander ES, Golub TR, Zick A, Orthwein A, Lawrence MS, Batra RN, Caldas C, Haber DA, Laird PW, Shen H, Ellisen LW, D'Andrea AD, Chanock SJ, Foulkes WD*, **Getz G***. A mutational signature reveals alterations underlying deficient homologous recombination repair in breast cancer. *Nat Genet*. 2017 Aug 21.

Rheinbay E, Parasuraman P, Grimsby J, Tiao G, Engreitz JM, Kim J, Lawrence MS, Taylor-Weiner A, Rodriguez-Cuevas S, Rosenberg M, Hess J, Stewart C, Maruvka YE, Stojanov P, Cortes ML, Seepo S, Cibulskis C, Tracy A, Pugh TJ, Lee J, Zheng Z, Ellisen LW, Iafrate AJ, Boehm JS, Gabriel SB, Meyerson M, Golub TR, Baselga J, Hidalgo-Miranda A, Shioda T, Bernards A, Lander ES, **Getz G**. Recurrent and functional regulatory mutations in breast cancer. *Nature*. 2017 Jul 6;547(7661):55-60.

Kim J, Mouw KW, Polak P, Braunstein LZ, Kamburov A, Tiao G, Kwiatkowski DJ, Rosenberg JE, Van Allen EM, D'Andrea AD, **Getz G**. Somatic ERCC2 mutations are associated with a distinct genomic signature in urothelial tumors. *Nat Genet*. 2016. Jun; 48(6): 600-6.

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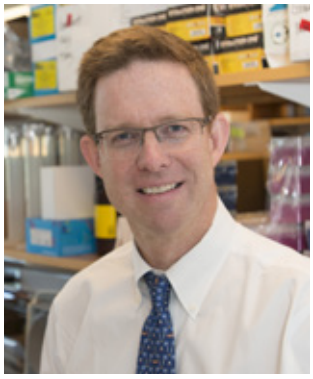
Lawrence MS, Stojanov P, Mermel CH, Robinson JT, Garraway LA, Golub TR, Meyerson M, Gabriel SB, Lander ES*, **Getz G***. Discovery and saturation analysis of cancer genes across 21 tumour types. *Nature*. 2014 Jan 23;505(7484):495-501.

*Co-corresponding authors



Timothy A. Graubert, MD

The Graubert laboratory focuses on the molecular basis of human blood cancers, including acute myeloid leukemia and myelodysplastic syndromes. The laboratory utilizes a variety of genomic platforms to interrogate primary samples from patients with myeloid malignancies to identify inherited and somatic mutations that drive these diseases. The goal of these studies is to gain insight into the biological basis of myeloid leukemias, and to improve strategies for diagnosis, risk stratification, and targeted therapy.

Graubert Laboratory

Amy Bertino, PhD
Timothy A. Graubert, MD
Wan Yee Leong, PhD
Sumit Rai, PhD
Pavan Reddy, PhD
Vineet Sharma, PhD

Clonal heterogeneity of myelodysplastic syndromes

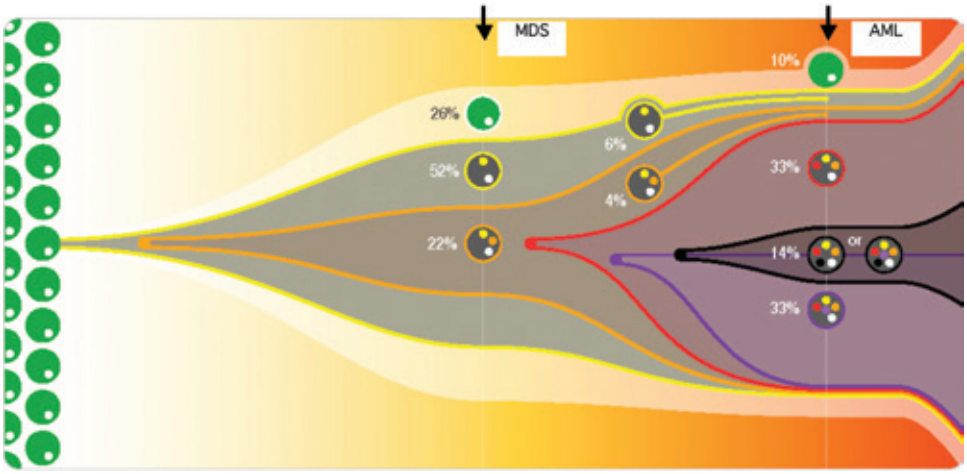
Myelodysplastic syndromes are the most common form of acquired bone marrow failure in adults. Despite the ineffective hematopoiesis that is characteristic of this disease in its early stages, we found through whole genome sequencing that nearly all cells in the bone marrow of these patients are clonally derived (see Figure). When patients evolve to acute myeloid leukemia (which occurs in approximately one third of cases), new subclonal populations emerge that are derived from the original ("founding") clone. These findings raise the possibility that the prognostic value of recurrent mutations in myelodysplastic syndrome and the efficacy of therapies that target these mutations may depend not only on the presence or absence of these mutations, but also on their position within the clonal hierarchy of this disease.

RNA splicing defects at the root of myelodysplastic syndromes

We and several other groups discovered recurrent somatic mutations in genes encoding core components of the RNA splicing complex (the "spliceosome") in patients with myelodysplastic syndrome. Mutations in this pathway tend to be mutually exclusive, suggesting that more than one splicing gene mutation in a cell provides no additional selective advantage, or is deleterious to the clone. We have focused on *U2AF1* which encodes a component of the U2 snRNP that binds to the AG dinucleotide at the 3′ intronic splice acceptor site. Mutations in U2AF1 arise early in the pathogenesis of myelodysplastic syndromes (in the founding clone) and affect almost exclusively two codons in predicted zinc finger domains. We have shown that the most common mutation (S34F) has gain-of-function activity in splicing assays. Current work in the Graubert laboratory is focused on comprehensive analysis of the impact of U2AF1 mutations on splicing, the functional consequences of these mutations for blood cell development, and vulnerabilities created by splicing gene mutations that provide opportunities for novel therapies.

Inherited predisposition to myelodysplastic syndrome/acute myeloid leukemia

Acute myeloid leukemia and myelodysplastic syndromes are usually sporadic, late-onset cancers, but in rare instances (<1%) these diseases aggregate in families. In these families, predisposition to acute myeloid leukemia/myelodysplastic syndrome may be a consequence of an inherited bone marrow failure syndrome, but in other cases these are highly penetrant, autosomal dominant, Mendelian disorders. Three genes (*RUNX1*,



Clonal evolution from myelodysplastic syndrome (MDS) to acute myeloid leukemia (AML). Whole genome sequencing at the time of MDS diagnosis (left arrow) in a representative patient identified a founding clone comprising ~52% of the bone marrow cellularity and a subclone derived from the founding clone in ~22% of cells. When this patient progressed to AML (right arrow), the original clones were still present and had spawned three new subclones that were dominant in the bone marrow at this time point.

GATA2, CEBPA) explain fewer than half of these Mendelian cases. The genetic basis in the majority of families is not yet known. Furthermore, the latency and incomplete penetrance of acute myeloid leukemia/myelodysplastic syndrome in mutation carriers suggest that acquisition of cooperating somatic mutations is required for malignant transformation. We have accumulated a large panel of samples from affected and unaffected members of these families. Ongoing studies in the Graubert laboratory are focused on identification of novel germline variants in families that lack known predisposing factors, and characterization of the landscape of cooperating somatic mutations that arise in these cases. This information is important for genetic counseling in these families, for selection of optimal bone marrow transplant donors, and to increase our understanding of the biological basis of acute myeloid leukemia and myelodysplastic syndromes.

Selected Publications:

Nguyen HD, Leong WY, Li W, Reddy PNG, Sullivan JD, Walter MJ, Zou L, **Graubert TA**. Spliceosome Mutations Induce R loop-Associated Sensitivity to ATR Inhibition in Myelodysplastic Syndrome. *Cancer Research*. 2018 Jul 27.

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Saez B, Walter MJ, **Graubert TA**. Splicing factor gene mutations in hematologic malignancies. *Blood,* 2017 Mar 9;10(129): 1260-1269.

Shirai CL, White BS, Tripathi M, Tapia R, Ley JN, Ndonwi M, Kim S, Shao J, Carver A, Saez B, Fulton RS, Fronick C, O'Laughlin M, Lagisetti C, Webb TR, **Graubert TA**, Walter MJ. Mutant U2AF1-Expressing Cells Are Sensitive to Pharmacological Modulation of the Spliceosome. *Nat. Communications*, 2017 Jan 9, 8:14060.

Okeyo-Owuor T, White BS, Chatrikhi R, Mohan DR, Kim S, Griffith M, Ding L, Ketkar-Kulkarni S, Hundal J, Laird KM, Kielkopf CL, Ley TJ, Walter MJ, **Graubert TA**. U2AF1 mutations alter sequence specificity of pre-mRNA binding and splicing. *Leukemia*. 2015 Apr;29(4):909-17.

Wilhelm Haas, PhD

The Haas laboratory uses quantitative mass spectrometry-based proteomics to characterize cancer cells and their vulnerabilities in a comprehensive proteome-wide manner. This is fueled by recent discoveries that have enhanced the depth and throughput of proteomics in quantifying proteins and their post-translational modification. These improvements have put us at a pivotal point in the field of mass spectrometry, where, for the first time, we are able to handle the analysis of the large number of samples that have to be examined to generate the basis for understanding a disease that displays the heterogeneity found in cancer. We are specifically interested in mapping changes in the global landscape of protein-protein interactions - the interactome - that occur in cancer cells, and we have shown that dysregulations in the interactome are enabling the prediction of cancer vulnerabilities. We believe that our proteomics technologies have the potential to become a powerful tool in basic and clinical cancer research and may be used to diagnose cancer, predict its susceptibility, and monitor its progression.

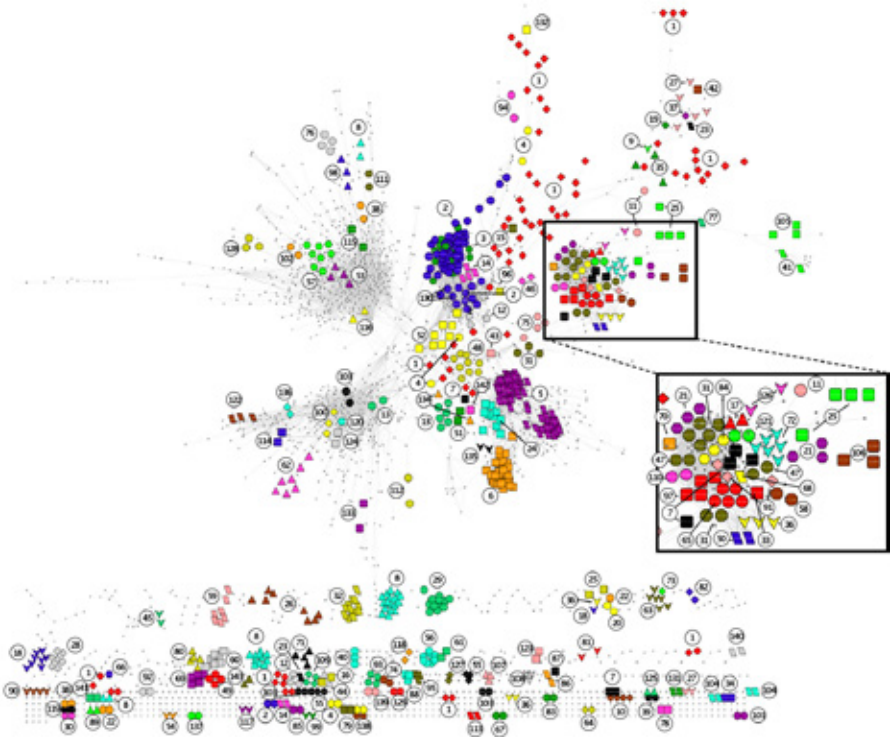
Haas Laboratory

Ridwan Ahmad
Wilhelm Haas, PhD
Meena Kathiresan, PhD
Ashok Khatri, MS
Johannes Kreuzer, PhD
Robert Morris, PhD

Cancer is based on dynamic changes of the genome that ultimately translate into an altered proteome, optimized for uncontrolled cell growth and division. In addition, many pathways, initially causing cancer further promote the propagation of altered genetic information, accelerating the adaption of cancer cells to new environments. This dynamic process becomes even more complex if taking into account the dynamic state of the cellular proteome that is regulated by protein synthesis and degradation, posttranslational modifications, protein localization, and the interaction of proteins with other proteins as well as with different classes of biomolecules. While the "cancer genome" can now be easily accessed due to advances in DNA sequencing technology, the information contained in the "cancer proteome" has remained largely untapped due to technical challenges in quantifying the large number of proteins expressed in mammalian cells.

Yet, the proteome holds enormous potential to improve our understanding of the basic principles underlying cancer to revolutionize the early diagnosis of the disease and to improve patient care. Up to date, virtually all targeted therapeutics in cancer treatment are targeting proteins. Understanding how these drugs alter the proteome and the interactome – the global map of protein-protein interactions – has the potential to help us refine our approaches to drug design.

The core technology used in our research group is high-throughput quantitative proteomics enabled through multiplexed mass spectrometry. This technology allows us to map the proteome of a cancer cell line or tumor tissue at high throughput. Analyzing the proteome maps across a panel of cancer cell lines, we recently made the observation that the concentration of proteins in known complexes are accurately correlated across all analyzed cell lines. We showed that



A Map of Protein-Protein Interactions Identified Using the IMAHP Technology Based on Protein Concentration Co-Regulation across Cancer Cell Lines.

protein co-regulation analysis allows the genome-wide mapping of protein-protein interactions with an accuracy ten-times larger than when using co-expression analysis based on RNAseq data. We further found that deviations from co-regulation of two interacting proteins in specific cancer cell lines reflect perturbed cellular circuitry, and it remarkably predicts sensitization to therapeutics targeting regulatory modules in the associated pathway. We have termed this approach to fast, in-depth characterization of protein-protein interaction landscapes the Interactome MApping by High-throughput quantitative Proteome analysis (IMAHP) technology. This novel method has been developed in collaboration with the laboratory of Cyril Benes at the MGH Cancer Center. It enables an interactome-wide mapping of protein-protein interaction dysregulation and inferred cancer vulnerabilities of any cancer sample based on a proteome map that is acquired at high throughput.

We are further interested in the development and application of high-throughput proteomics methods to globally map protein phosphorylation dynamics in cancer samples and to use the data to specifically identify new kinase targets as cancer vulnerabilities.

Our goals are to apply this technologies to (i) identify novel cancer vulnerabilities that direct new treatment strategies, to (ii) map cancer vulnerability dynamics, such as those occurring in the development of therapy resistance, to identify novel targets that enable to overcome the treatment resistance, and to (iii) use our technology in a clinical setting for mapping tumor vulnerabilities to inform treatment strategies in a patient-specific manner.

Selected Publications:

Lapek JD Jr, Greninger P, Morris R, Amzallag A, Pruteanu-Malinici I, Benes CH*, **Haas W***. Detection of dysregulated protein-association networks by high-throughput proteomics predicts cancer vulnerabilities. *Nat. Biotechnol.* 2017; 35, 983-989.

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McAlister GC, Nusinow DP, Jedrychowski MP, Wühr M, Huttlin EL, Erickson BK, Rad R, **Haas W**, Gygi SP. MultiNotch MS3 enables accurate, sensitive, and multiplexed detection of differential expression across cancer cell line proteomes. *Anal Chem.* 2014; 86, 7150-7158.

Ting L, Rad R, Gygi SP*, **Haas W***. MS3 eliminates ratio distortion in isobaric multiplexed quantitative proteomics, *Nat. Methods.* 2011; 8, 937-940.

*Co-corresponding authors

Daniel Haber, MD, PhD

The Haber laboratory focuses on understanding the fundamental genetics of human cancer, from inherited mutations that confer familial predisposition to genetic mutations that are acquired by tumors themselves and may render them susceptible to specific targeted drug therapies. For example, we have identified mutations in the EGFR gene that confer dramatic sensitivity of some lung cancers to drugs that inhibit that pathway, pointing toward the importance of genetic classification of common epithelial cancers in applying novel targeted therapies. We have also collaborated with the bioengineering team led by Dr. Mehmet Toner, the molecular biology group of Dr. Shyamala Maheswaran, and the Massachusetts General Hospital (MGH) Cancer Center clinical disease centers to develop, characterize and apply a microfluidic device capable of isolating rare circulating tumor cells (CTCs) in the blood of patients with cancer. This new technology offers the promise of 1) noninvasive monitoring of cancers during their treatment for the emergence of drug resistance; 2) early detection of invasive cancers; and ultimately 3) understanding and preventing blood-borne spread of cancer.

Haber Laboratory*

Risa Burr, PhD
Brian Chirn
Christina Costantino
Valentine Comaills, PhD
Taronish Dubash, PhD
Richard Ebright**
Hongshan Guo, PhD
Daniel Haber, MD, PhD
Uyen Ho
Xin Hong, PhD
Elad Horwitz, PhD
Mark Kalinich**
Selena Li**
Laura Libby
Satoru Matsuda, MD**
Doug Micalizzi, MD
Stefanie Morgan, PhD
Kira Niederhoffer
Benjamin Nicholson
Kira Niederhoffer
Brittany Reeves
Yongheng Wang
Benjamin Wesley
Devon Wiley
Ben Wittner, PhD

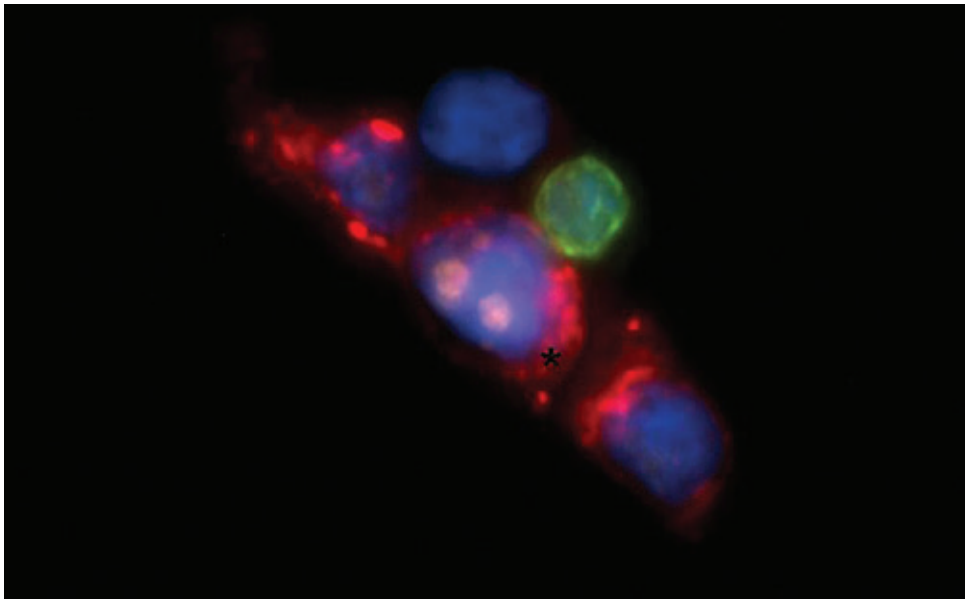
* Co-directed with Shyamala Maheswaran, PhD

** Graduate students

Our laboratory is interested in the genetics of human cancer. Current projects include the use of a microfluidic device to capture circulating tumor cells (CTCs) and its application in early detection of invasive cancer, molecular-directed therapy, and in the study of human cancer metastasis.

Circulating Tumor Cells and Molecular Genetics Underlying Targeted Cancer Therapeutics

Activating mutations in the epidermal growth factor receptor (*EGFR*) were identified in our laboratory in the subset of non-small cell lung cancer (NSCLC) with dramatic responses to the tyrosine kinase inhibitor gefitinib. We have studied mechanisms underlying such oncogene addiction, as well as the pathways that lead to the acquisition of resistance to targeted therapies, including the application of irreversible kinase inhibitors to circumvent mutations that alter drug binding affinity. Following these efforts to monitor the emergence of drug resistance mutations, we established collaborations with the Toner and Maheswaran laboratories to characterize novel microfluidic devices capable of isolating CTCs from the blood of cancer patients. Our most advanced version of these CTC-Chips relies upon blood flow through a specialized chamber, which allows the high efficiency depletion of antibody-tagged leukocytes, thereby enriching for intact CTCs without selection bias. We have shown that the number of captured CTCs correlates with clinical evidence of tumor response, and that the cells can be used to define molecular markers characteristic of the underlying malignancy, including *EGFR* mutations in lung cancer and measurements of androgen receptor (AR) activity in prostate cancer. We have applied next generation single-molecule RNA sequencing and RNA-in-situ hybridization to characterize



Circulating prostate tumor cell cluster stained for PSA (green) along with Ki67 (orange) and CD45 (red).

the heterogeneous expression profiles of individual CTCs in breast, prostate and pancreatic cancers, as well as melanoma and glioblastoma. To facilitate CTC quantitation and provide the sensitivity and specificity required for early cancer detection, we have established a droplet digital PCR readout for CTC-derived RNA, with promising applications in the early detection of liver cancer.

In addition to noninvasive detecting and monitoring of cancer, CTCs provide a window to study the process of blood-borne metastasis. We demonstrated treatment-associated epithelial-to-mesenchymal transitions (EMT) within CTCs from women with breast cancer. Using a combination of mouse models and patient-derived studies, we observed that tumor-derived fragments generate CTC-Clusters, which have greatly enhanced metastatic propensity compared with single CTCs. CTC-Clusters are held together by plakoglobin, whose knockdown dramatically suppresses CTC-Cluster formation and metastatic spread of breast cancer cells. We successfully established long-term *in vitro* cultures of CTCs from patients with estrogen-receptor (ER)-positive breast cancer, identifying treatment-associated mutations in the estrogen receptor (ESR1), as well as acquired mutations in drugable therapeutic targets, such as *PIK3CA* and *FGFR*. The development of such CTC-derived cultures may enable functional predictive drug testing, combined with detailed genetic analysis of tumor cells sampled noninvasively during the course of cancer treatment. In cultured CTCs from women with advanced ER+ breast cancer, we documented dramatic plasticity, with a proliferative HER2-expressing subpopulation interconverting spontaneously with a drug-resistant Notch1-expressing subset. Using mouse reconstitution models, we demonstrated the consequences of this phenotype switch for both tumorigenesis and drug response. Ongoing studies are directed at using patient-derived CTCs and mouse models to understand key steps in cancer metastasis, including the shift from cell quiescence to proliferation, viability during blood-borne transit, and resistance to targeted and immune therapies.

Selected Publications:

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*Co-corresponding authors

Nir Hacohen, PhD

The Hacohen laboratory consists of immunologists, geneticists, biochemists, technologists and computational biologists working together to develop new and unbiased strategies to understand basic immune processes and immune-mediated diseases, with an emphasis on the innate immune system and personal medicine. We address three key questions in immunology (1) how are immune responses against cancer initiated, maintained and evaded? (2) what are the immune circuits that sense and control pathogens, such as viruses and bacteria? (3) how does immunity against the body develop, in particular, in patients with autoimmune lupus? In addition to discovering and studying specific molecular and cellular mechanisms, we also address how and why the immune response (to tumors, pathogens or self) varies so dramatically across individuals. Finally, we are adapting our unbiased analytical strategies into real-world therapeutics, having initiated clinical trials (with our collaborator Dr. Catherine Wu), in which patients are vaccinated against their own tumors with a fully personal vaccine that is designed based on a computational analysis of their personal tumor genome.

Hacohen Laboratory

Arnon Arazi, PhD
Matthew Bakalar, PhD
Stacey Bjorgaard
Jonathan Chen, MD, PhD
Bing Shao Chia*
Ang Cui*, MS
Johanna Dahlqvist, MD, PhD
Thomas Eisenhaure
Nir Hacohen, PhD
Paul Hoover, MD, PhD
Alice Yuk Lan, PhD
Bo Li*
David Lieb, MS
Bingxu Liu*
Karin Pelka, PhD
Karen Rahman
John Ray, PhD
Raktima Raychowdhury, PhD
Miguel Reyes*
Moshe Sade-Feldman, PhD
Larry Schweitzer, PhD
Sisi Sarkizova*, MS

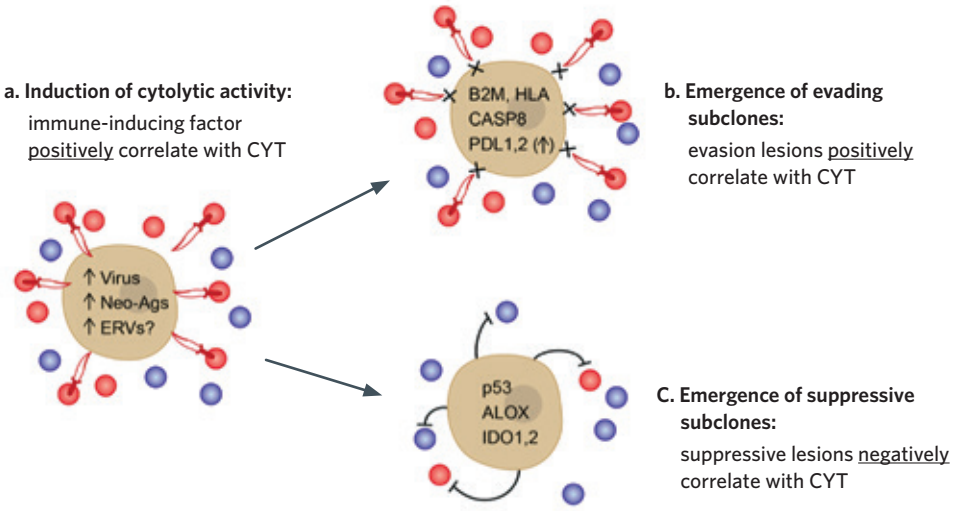
* PhD Candidates

Initiators, resistors and targets of tumor immunity

While cancer immunology has been deeply studied in animal models, there remain many open questions in human tumor immunology due to lack of tools to investigate human samples. We have developed genetic and genomics approaches to explain the large variance in anti-tumor immunity across people, and to discover how tumors evolve to resist productive immunity. We recently found that one of the best predictors of anti-tumor immunity is the load of neoantigens (mutated peptides presented on the surface of tumor cells on HLA molecules, *Blood* 2014); we also identified somatic mutations in tumors that induce or resist anti-tumor immunity in patients (Rooney et al., *Cell* 2015). We have also developed new methods to predict somatic mutations that generate presented antigens (Abelin et al, *Immunity* 2017). These studies have been leading to novel therapeutic approaches and targets for immunotherapy. In particular, based on the finding that patients develop immunity against mutated neoantigens derived from their tumors (Hacohen et al., *Cancer Immunology* 2013; Rajasagi et al., *Blood* 2014), we have developed and tested a personal tumor vaccine targeting multiple HLA-associated neoantigens in human tumors (together with Dr. Catherine Wu at DFCI, Ott et al., *Nature* 2017).

Genes and networks underlying host-pathogen interactions

We have developed a set of integrative strategies to dissect networks of genes involved in sensing or controlling pathogens. We identified host pathways supporting or restricting influenza such as IFITM3 (Shapira et al., *Cell* 2009), transcription factors and signaling molecules mediating the innate immune responses to viruses and bacteria (Amit et al., *Science* 2009; Chevrier et al., *Cell* 2011), and components of innate DNA sensing (Lee et al., *Nat Immun* 2013).



A model for tumor-immune co-evolution by which: (a) intrinsic tumor factors -- such as mutated neoantigens, viruses or endogenous retroviruses -- induce local immune infiltrates (blue circles) that include cytolytic effector cells (CYT=cells expressing GZMA/PRF1*; red circles) that kill tumors (daggers); (b) under pressure from cytolytic immune cells, tumor subclones are selected for resistance mutations (within the genes indicated) that autonomously evade killing or (c) non-autonomously suppress the immune infiltrate.*

Most recently, we demonstrated that genome-wide CRISPR screens effectively discover genes involved in sensing pathogens (Parnas et al., *Cell* 2015), and are now using this system to discover genes involved in sensing diverse pathogens and controlling viral infections.

Genetic basis for inter-individual variations in immune responses

We have also developed genomic strategies to analyze human immune responses and explain immune phenotypes with germline genotypes. We discovered the genetic basis for inter-individual variation in the innate immune response to viruses and bacteria (Lee et al., *Science* 2014; Raj et al., *Science* 2014; Ye et al., *Science* 2014). For example, we found that common alleles of IRF7 tune the strength of an individual's anti-viral response. Building on these studies, we have recently developed and are using systematic methods to analyze the role of genetic and non-genetic variations in human immunity and their impact on autoimmune diseases.

Innate immune drivers of autoimmunity

Deficiencies in nucleases that degrade DNA lead to accumulation of self DNA, activation of innate immune responses and development of autoimmune disorders, including systemic lupus erythematosus and Aicardi-Goutières syndrome in humans, and autoimmune arthritis, nephritis and myocarditis in mice. We have been interested in understanding how autoimmunity develops upon triggering of innate immunity by self DNA (rather than pathogen-derived DNA). In studying this question, we made the surprising observation that immunostimulatory DNA can arise from host damaged DNA that is exported from the nucleus to the lysosome (Lan et al., *Cell Rep* 2014). We hypothesize that this cellular process is a source of inflammation in autoimmunity, cancer, chemotherapy and aging. We also developed an integrated proteomic and genomic approach to uncover novel factors and small molecules targeting this pathway that may be useful to treat these diseases (Lee et al., *Nat Imm* 2013). To deepen our understanding of DNA and RNA pathways that drive autoimmunity, we are currently analyzing immune responses in lupus nephritis patients, with an emphasis on cellular and molecular analysis of kidney biopsies and blood samples from lupus patients.

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Parnas O*, Jovanovic M*, Eisenhaure TM*, Herbst RH, Dixit A, Ye C, Przybylski D, Platt RJ, Tirosh I, Sanjana NE, Shalem S, Satija R, Raychowdhury R, Mertins P, Carr SA, Zhang F, **Hacohen N***, Regev A*. A Genome-wide CRISPR Screen in Primary Immune Cells to Dissect Regulatory Networks. Cell. 2015 Jul 30;162(3):675-86.

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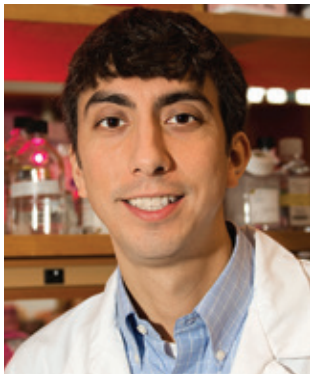
*Equal contribution



Aaron Hata MD PhD

The research goal of **the Hata laboratory** is to advance targeted therapies to benefit patients with lung cancer. Our research focuses on understanding the biological underpinnings of sensitivity and resistance to kinase inhibitor targeted therapies in lung cancers with specific genetic abnormalities (EGFR mutations, ALK translocations, KRAS mutations, etc.). In particular, we seek to understand how kinase inhibitors modulate signaling networks that regulate cancer cell growth and survival, and characterize the molecular mechanisms of acquired resistance to these agents. More recently, we have begun to focus on understanding how cancer cells adapt and evolve during the course of therapy in order to identify vulnerabilities of drug tolerant cancer cells that might be exploited to prevent resistance from developing. Our studies are highly translational, combining cell culture models, patient-derived mouse (PDX) models and assessment of clinical specimens, and are performed in close collaboration with clinicians in the Thoracic Oncology group.

Hata Laboratory

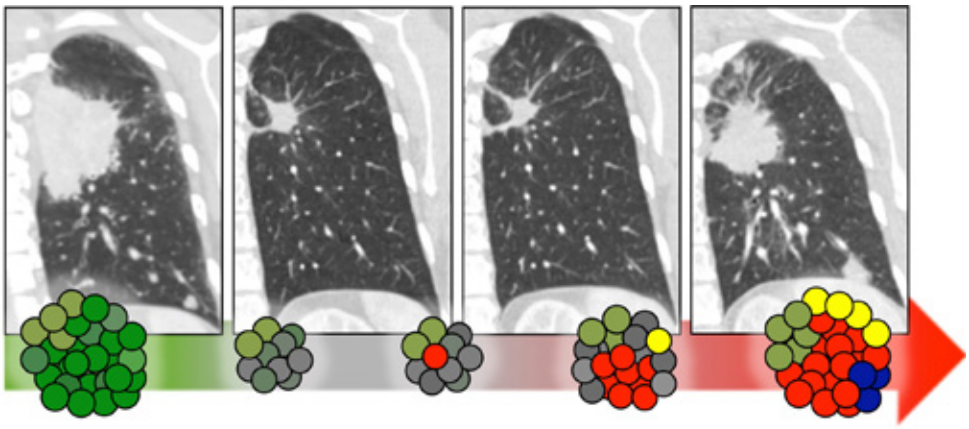
Kankana Bardhan, PhD
Heidie Frisco-Cabanos, PhD
Aaron Hata, MD PhD
Haichuan Hu, MD
Hideko Isozaki, PhD
Chendi Li, PhD
Lia Limone
Philicia Moonsamy, MD
Varuna Nangia
Audris Oh
Kylie Pruisto-Chang
Kathy Shaw
Li Wan
Satoshi Yoda, MD

EGFR

EGFR inhibitors have revolutionized the treatment of EGFR mutant non-small cell lung cancer (NSCLC), with patients achieving robust responses. Unfortunately, drug resistance invariably occurs, and patients typically relapse after one year of treatment. Next generation EGFR inhibitors that overcome the most common resistance mutation, $EGFR^{T790M}$, have now entered the clinic, but ultimately resistance to these agents also occurs. We recently demonstrated that resistance due to acquisition of the $EGFR^{T790M}$ mutation can arise via evolution of drug tolerant clones that survive initial therapy and then acquire the mutation. This suggests that drug tolerant cells that survive initial EGFR inhibitor therapy may comprise a cellular reservoir from which heterogeneous mechanisms of resistance may arise. We are currently performing single cell analysis and multi-dimensional profiling of patient tumor specimens and PDX models before, during and after treatment in order to understand the genetic, epigenetic and micro-environmental mechanisms that contribute to the evolution of acquired drug resistance *in vivo*. By identifying vulnerabilities of drug tolerant cells prior to development of resistance, we hope to develop novel therapeutic strategies that will disrupt this perpetual cycle of acquired resistance.

ALK

Anaplastic lymphoma kinase *(ALK)* gene rearrangements have emerged as well-established oncogenic drivers and therapeutic targets in NSCLC. Several successive generations of ALK inhibitors have now entered the clinic, and ALK-dependent and ALK-independent resistance mechanisms have been identified. By interrogating *in vitro* and *in vivo* models of acquired resistance to ALK inhibitors, including cell lines and PDX models established from biopsies of patients at the time of disease progression,



EGFR mutant lung cancers can develop acquired resistance to EGFR inhibitors (e.g. acquisition of the gatekeeper EGFR^{T790M} mutation) by selection of pre-existing EGFR^{T790M} cells, or via evolution of initially EGFR^{T790M}-negative drug tolerant cells that then develop the mutation during the course of treatment. EGFRi denotes EGFR inhibitor treatment, such as gefitinib or erlotinib. Reproduced from Hata and Niederst, et al. Nature Medicine 2016.

we are uncovering novel mechanisms of acquired resistance and developing treatment strategies to overcome them. Additionally, we are interested in understanding the impact of intra- and intertumoral heterogeneity on drug response and acquired resistance to ALK inhibitors. By monitoring clonal evolution over time through analysis of serial tumor biopsies and circulating cell free tumor DNA, we hope to better tailor ALK inhibitor therapies to individual patients.

KRAS

KRAS is the most common driver oncogene in lung cancer and development of therapeutic strategies to improve the survival of these patients represents one of the most important needs in all of oncology. We are exploring multiple strategies to develop effective therapies for KRAS mutant lung cancer. First, we are testing novel agents that directly inhibit mutant KRAS. Second, we are exploring how drugs that directly target apoptotic regulators such as BCL-2 family proteins may enhance the efficacy of kinase inhibitors to induce apoptosis in KRAS mutant lung cancer. Third, we are exploring how targeted therapies and immunotherapies can be integrated in order to induce long term remissions in patients with KRAS mutant lung cancer. We believe this latter approach will set a new standard for understanding how interactions between oncoprotein-activated pathways and the immune microenvironment regulate tumor growth.

Selected Publications:

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Konrad Hochedlinger, PhD

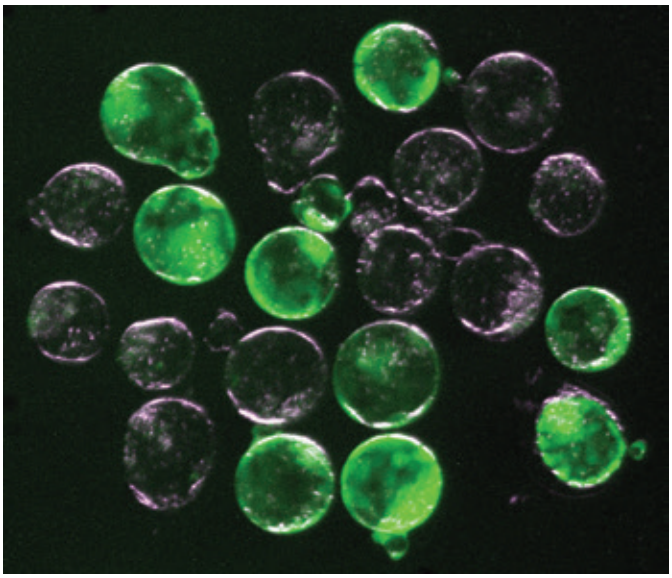
The Hochedlinger laboratory explores the molecular mechanisms underlying pluripotency, which is the ability to produce all mature cell types of the body. Previous groundbreaking discoveries have shown that adult cells can be reprogrammed into pluripotent stem cells by activating a handful of embryonic genes. The resultant cells, called induced pluripotent stem cells (iPSCs), have tremendous therapeutic potential; they can be derived from any patient's skin or blood cells. In the laboratory, iPSCs can be coaxed into many specialized cell types. Our lab has contributed to a better understanding of the process of cellular reprogramming, which allowed us to elucidate basic mechanisms that maintain cellular identity and prevent aberrant cell fate change. Our ultimate goal is to utilize these mechanistic insights for the development of new strategies to treat cancer and other complex diseases.

Hochedlinger Laboratory

Justin Brumbaugh, PhD
Amy Coffey, MS
Bruno Di Stefano, PhD
Yvonne Hernandez
Konrad Hochedlinger, PhD
Michael Hoetker, MD
Aaron Huebner, PhD
Masaki Yagi, PhD

The Hochedlinger lab is studying the mechanisms of cellular reprogramming using transcription-factor-mediated conversion of somatic cells into induced pluripotent stem (iPSCs). iPSCs are typically derived by retroviral transduction of the embryonic transcription factors Oct4, Sox2, c-Myc and Klf4, which reset the differentiation state of an adult cell into that of a pluripotent cell. The underlying transcriptional and epigenetic changes remain largely elusive due to the low efficiency of reprogramming and the heterogeneity of cell cultures. Importantly, iPSCs have been derived from different species—including human patients—and therefore provide a unique platform to model degenerative disorders such as Alzheimer's disease, Parkinson's disease and diabetes. Moreover, iPSCs could be ultimately used in regenerative medicine to replace damaged cells and tissues with genetically matched cells.

We have identified biomarkers to track and prospectively isolate rare intermediate cell populations that are poised to become iPSCs, and we are currently using these populations to understand the transcriptional, epigenetic and proteomic changes in cells undergoing reprogramming. In addition, we have shown that terminally differentiated beta cells and lymphocytes can be reprogrammed into iPSCs, thus demonstrating that induced pluripotency is not limited to rare adult stem cells as has originally been suggested. Nevertheless, we discovered that immature hematopoietic cells give rise to iPSCs more efficiently than any tested mature cell types, suggesting that the differentiation stage and therefore the epigenetic state of the starting cell has a profound effect on its potential to be reprogrammed. At the molecular level, we have identified the p53 and p16/p19 tumor suppressor pathways as well as the Tgf-beta signaling cascade as roadblocks during the reprogramming process, pointing out striking similarities between pluripotent cells and cancer cells. Additionally, our lab has conducted unbiased shRNA screens for barriers to reprogramming, uncovering new mechanisms that safeguard somatic cell identity. For example, we identified the histone



Induced myogenic progenitor cells (iMPCs) derived from fibroblasts. Immunostaining for markers of muscle stem cells (Pax7, red) and differentiated cells (MyoD, green; MyHC, purple)(see Bar-Nur et al., Stem Cell Reports 2018 May 8;10(5):1505-1521).

Image: Ori Bar-Nur, PhD

chaperone CAF-1 and the protein modifier Sumo2 as novel modulators of mammalian cell fate change and we are currently exploring the underlying mechanisms as well as their role in tissue homeostasis and cancer.

One major roadblock for the therapeutic use of iPSCs has been the fact that integrating viruses were initially used to deliver the reprogramming genes to cells, resulting in genetically altered iPSCs. By using adenoviruses expressing the reprogramming factors transiently in cells, we were able to produce the first iPSCs devoid of any viral elements and thus any genetic manipulation. In addition to eliminating a potential roadblock to the therapeutic application of iPSC technology, this finding allowed us to compare unaltered iPSCs to genetically matched embryonic stem cells, which represent the gold standard for pluripotent stem cells. For example, we discovered that the Dlk1- Dio3 imprinted gene cluster is aberrantly silenced by hypermethylation in many iPSC lines, which correlates with their impaired developmental potential. We recently extended this study to human cells, demonstrating that isogenic, vector-free iPSCs are equivalent to human embryonic stem cells. These results indicated that genetic background and reprogramming method are major contributors of transcriptional and epigenetic variation in human pluripotent stem cell lines.

In a separate line of investigation, we are studying the role of Sox2 in adult tissues. While Sox2 has been mostly interrogated in the context of pluripotent stem cells and cellular reprogramming, recent data suggest that it may play important functions in adult tissues as well. For example, Sox2 is essential for neural stem cell maintenance, and its coding region is amplified in lung and esophageal cancer, thus implicating Sox2 in adult tissue regeneration and tumorigenesis. We have identified Sox2-expressing cells in several adult tissues where it has not previously been characterized, including squamous epithelia lining the stomach, anus and cervix as well as in testes, lens and glandular stomach. Unexpectedly, we discovered that Sox2 functions as a tumor suppressor in a mouse model of stomach cancer, which may have important therapeutic implications given its role as an oncogene in other tissues. Future work in the lab is aimed at further understanding the molecular and functional role of Sox2 and Sox2+ cells in stomach homeostasis and using mouse and human cells.

Selected Publications:

Di Stefano B, Ueda M, Sabri S, Brumbaugh J, Huebner AJ, Sahakyan A, Clement K, Clowers KJ, Erickson AR, Shioda K, Gygi SP, Gu H, Shioda T, Meissner A, Takashima Y, Plath K, **Hochedlinger K**. Reduced MEK inhibition preserves genomic stability in naive human embryonic stem cells. *Nat Methods*. 2018 Aug 20. doi: 10.1038/s41592-018-0104-1. [Epub ahead of print]

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Choi J, Huebner AJ, Clement C, Walsh RM, Savol A, Lin K, Gu H, Di Stefano B, Brumbaugh J, Kim SY, Sharif J, Rose CM, Mohammad A, Odajima J, Charron J, Shioda T, Gnirke A, Gygi SP, Koseki H, Sadreyev R, Xiao A, Meissner A & **Hochedlinger K**. Prolonged Mek1/2 suppression impairs the developmental potential of embryonic stem cells. *Nature*. 2017 Aug 10;548(7666):219-223.

Hanno Hock, MD, PhD

The Hock laboratory explores the molecular basis of blood cell formation and the pathogenesis of leukemia and lymphoma. Specifically, we study the transcription factors that regulate gene activity during normal blood cell development and how the transcriptional apparatus goes awry in cancer. For example, we have developed important insights into a network of transcription factors that help maintain blood stem cells in the bone marrow; this work could lead to new strategies for increasing the yield of stem cells for bone marrow transplantation. Another project in our laboratory focuses on deciphering the multistep process that leads to lymphoblastic leukemia of childhood, with the goal of identifying new drug targets for this devastating disease. Finally, we are interested in how DNA packaging affects the interaction between genes and transcription factors, especially with regard to oncogenes and tumor suppressor genes important in human cancer.

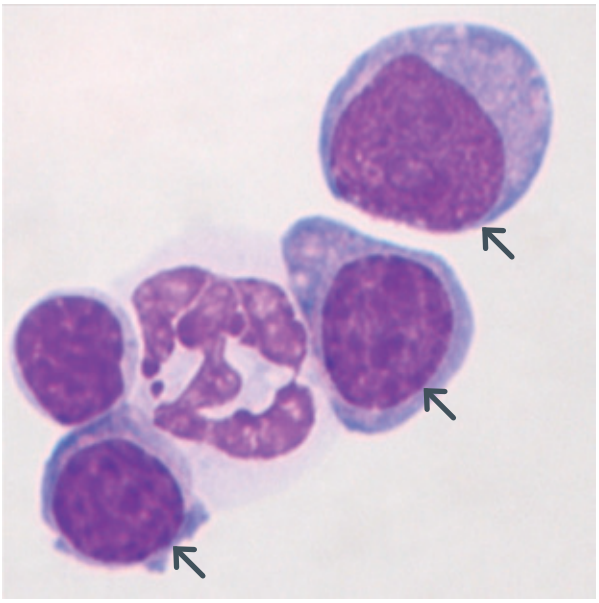
Hock Laboratory

Hanno Hock, MD, PhD
Daniel Kramer
Ondrej Krejci, PhD
Ryan LeGraw
Matthew Leon
Jeffrey Wolfe Schindler, PhD

Our laboratory is interested in the molecular control of normal and malignant stem cells with an emphasis on the hematopoietic system. Blood cells need to be continuously replenished by a small population of hematopoietic stem cells (HSCs) that have the capacity to both self-renew and mature stepwise into all known blood lineages. HSCs are also the ancestors of leukemia and lymphoma cells. As HSCs mature, they undergo successive changes in gene expression. The transcriptional apparatus must ensure that genes specific to immature cells are repressed as differentiation proceeds, while genes that are necessary for mature cells become activated. This activating and inactivating of genes is achieved by cooperative action of a variety of lineage-specific and general transcription factors and the complex molecular machinery that regulates the accessibility of different regions of the genome in chromatin. We investigate how transcription factors establish differentiation-specific transcriptional programs and how such programs can become derailed in cancer, leukemia and lymphoma.

Transcriptional control of normal and malignant hematopoietic stem cells in the adult bone marrow

Hematopoiesis in the bone marrow emanates HSCs. We are studying the basic biology of HSCs. Specifically we explore how a network of transcription factors that includes Tel- Etv6, Gfi1, Gfi1b and Gata2 maintains HSCs in the bone marrow (Hock et al. 2004, *Genes & Development*; Hock et al. 2004, *Nature*). The goal is to exploit the biology of transcriptional regulation of HSCs to maintain, expand, and possibly even generate HSCs ex vivo so that more patients will have the option of bone marrow transplantation. In a closely related effort, we are exploring the molecular programs of stem cells in leukemia and lymphoma to identify differences in their molecular regulation compared with



Dr. Hock's laboratory works on molecular mechanisms of normal differentiation and malignant transformation. The image shows normal blood cells and leukemic cells (arrows) from a novel experimental model generated in the lab.

normal HSCs. Such differences may allow us to specifically target tumor stem cells while sparing normal blood formation.

Deciphering the molecular events leading to acute lymphoblastic leukemia of childhood

About one in 2000 children develops this catastrophic illness, most often with a t(12;21) translocation. Despite very aggressive treatments, not all children can be cured, and some suffer from long-term side effects of their therapy. Rational development of more specific, less toxic treatments requires a precise understanding of the molecular mechanisms that cause the disease. We have discovered that TEL-AML1, the first hit in childhood leukemia, generates a preleukemic, latent lesion in HSCs. We are now exploring how additional genetic hits cooperate to derail normal blood development and generate leukemia. Deciphering the multistep pathogenesis of this entity is likely to serve as a paradigm for the development of other malignant diseases.

Exploration of novel epigenetic regulators in stem cells

Our understanding of how specialized cells of the body establish their identity by regulating access to genes continues to increase. For example, a large fraction of the genes active in brain cells are inactive in blood cells and, therefore, are stored in a very dense, inaccessible state. As most molecules involved in the regulation of gene accessibility have only recently been identified, studying their biology is likely to provide unique opportunities for the development of entirely novel therapies. We are investigating the utility of a group of proteins termed MBT-proteins, which is very important for condensing DNA and modifying histones. Evidence suggests that this protein family may play important roles in normal and malignant blood formation, but its precise functions remain poorly understood. Our laboratory has recently discovered an entirely novel, essential function of the family member L3mbtl2 in pluripotent stem cells.

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Eminli S, Foudi A, Stadtfeld M, Maherali N, Ahfeldt T, Mostoslavsky G, **Hock H**, Hochedlinger K. Differentiation stage determines potential of hematopoietic cells for reprogramming into induced pluripotent stem cells. *Nat Genet*. 2009; Sep, 41(9):968-76.

Jonathan G. Hoggatt, PhD

The Hoggatt laboratory is broadly interested in the stem cell niche regulatory mechanisms that govern tissue regeneration, particularly regulation by macrophages, and we have a specific interest in translational science for bone marrow transplantation and other treatments. Our laboratory identified a unique "highly engraftable" hematopoietic stem cell that we are currently investigating which has applications for further probing of stem cell niche biology, and clinical applications in transplantation, gene therapy, and other fields. We have also developed unique genetic mouse models allowing us to dynamically explore macrophage heterogeneity in a variety of disease settings.

• • •

Hoggatt Laboratory

Bin-Kuan Chou, PhD
Shruti Datari
Jonathan G. Hoggatt, PhD

Macrophage Regulation of Tissue Regeneration

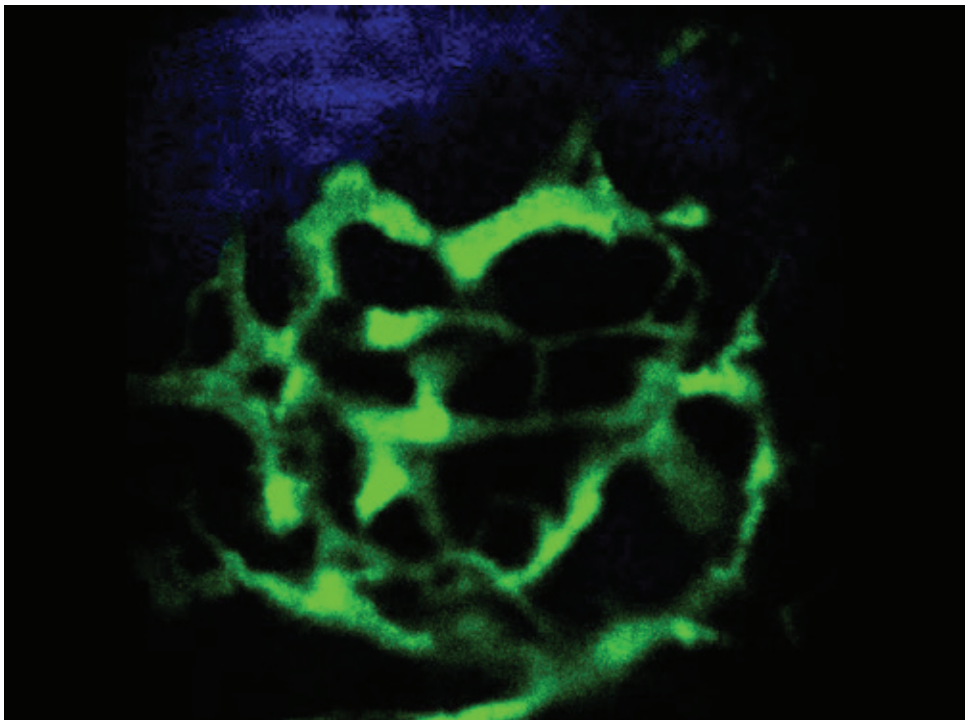
Macrophages are ancient cells of the innate and adaptive immune system. My old microbiology textbook defines macrophages as "scavengers and sentries – routinely phagocytizing dead cells and debris, but always on the lookout, ready to destroy invaders, and able to call in reinforcements when needed." Our laboratory believes they are so much more.

Tissue resident macrophage populations exist in virtually every tissue, whether they are Kupffer cells in the liver, alveolar macrophages in the lung, microglia in the brain or Langerhans cells in the skin. Some of these macrophages have been recently reported to specify hepatic progenitor cell fate, regulate epithelial progenitor niches in the colon and drive oligodendrocyte differentiation during remyelination in the central nervous system. After depletion of macrophages, an adult salamander is unable to regenerate an amputated limb. However, when macrophage levels were allowed to replenish, full limb regeneration capacity of failed stumps was restored upon re-amputation. Macrophages, therefore, may be a common cellular regulator across a diverse repertoire of stem cell niches. The problem that exists today is that macrophages are extraordinarily diverse and plastic, necessitating the need to identify specific subsets responsible for stem cell and tissue regeneration, in both homeostatic and disease scenarios.

We have created a unique mouse model that allows tracking of macrophages with deferring embryonic origins with specific, genetic-fluorescent markers, aiding in de-convoluting this heterogeneous cell population. Our laboratory is exploring several clinically relevant applications for stem cell transplantation, and will broadly use these macrophage tools and knowledge to delineate macrophage regenerative signals in multiple tissue stem cell niches, organ transplantation, and disease.

Hematopoietic Stem Cell Biology

Hematopoietic stem cell (HSC) transplantation is used to treat a number of malignant and non-malignant diseases. Over the last decade, there has been increasing evidence that the HSC pool is heterogeneous in function; with identification of HSCs with differing lineage outputs,



Shown are sinusoidal vessels (green) within the calvaria bone of mice during live, in vivo imaging of the hematopoietic stem cell niche.

kinetics of repopulation, length of life-span, and perhaps differences amongst HSCs contributing to homeostatic blood production from those that are the engraftable units in transplantation. Delineating the mechanisms of these functional differences has the potential to increase the efficacy of stem cell transplantation.

Currently, there are no great methods for prospectively isolating differing HSC populations to study heterogeneity; much of the data that has been acquired is based on clonal tracking, single cell transplantation, etc. We have developed a rapid mobilization regimen as a new method to acquire HSCs. Fifteen minutes after administering a single subcutaneous injection in mice, stem cell mobilization to the blood is greater than five days of granulocyte-colony stimulating factor (G-CSF) treatment; the current gold standard for hematopoietic mobilization. Surprisingly, when equivalent numbers of highly-purified HSCs from the blood of mice treated with the rapid regimen versus G-CSF were subsequently competitively transplanted into lethally irradiated recipients, the HSCs mobilized by the rapid regimen substantially outperformed those mobilized by G-CSF. The rapid regimen mobilizes a "highly engraftable" hematopoietic stem cell (heHSC) compared to those mobilized by G-CSF.

Much like panning for gold, we have used the differential mobilization properties of our regimen and G-CSF as a "biologic sieve" to isolate the heterogeneous HSC populations from the blood. Our laboratory will continue to leverage this approach to analyze the transcriptomic and epigenetic differences between the two populations of HSCs to determine the specific gene(s) that account for the heHSC phenotype, and to further explore the biologic potential of this new population of stem cells. These efforts have the potential to substantially increase our knowledge of heterogeneity and increase efficacy of HSC based clinical therapies.

Selected Publications:

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Palchaudhuri R, Saez B, **Hoggatt J**, Schajnovitz A, Sykes DB, Tate TA, Czechowicz A, Kfoury Y, Ruchika F, Rossi DJ, Verdine GL, Mansour MK, Scadden DT. *Nature Biotechnology*, 2016; Jul;34(7):738-45.

Hoggatt J, Kfoury Y and Scadden DT. Hematopoietic Stem Cell Niche in Health and Diseases. *Annual Review of Pathology*, 2016;11:555-581.

Hoggatt J[†*], Hoggatt AF[†*], Tate TA, Fortman J, Pelus LM*. Bleeding the Laboratory Mouse: Not All Methods are Equal. *Experimental Hematology*, 2016: Feb;44(2):132-137.

Hoggatt J, Tate TA and Pelus LM. Role of Lipegfilgrastim in the Management of Chemotherapy Induced Neutropenia. *International Journal of Nanomedicine*, 2015;10:2647-52.

*Co-corresponding authors

[†]Co-first authors

A. John Iafrate, MD, PhD

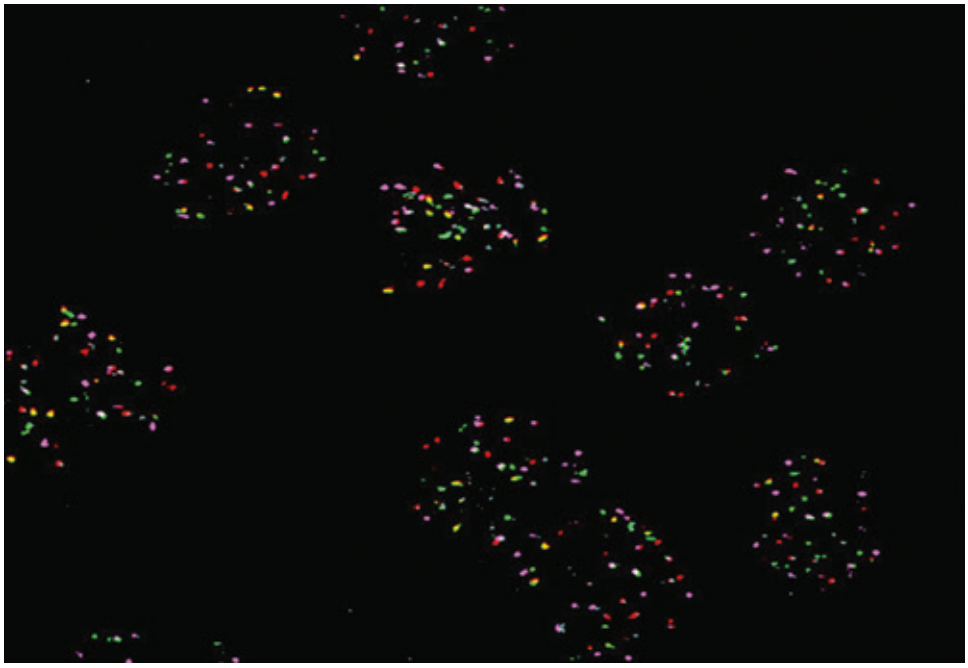
Research in **the Iafrate laboratory** focuses on bringing new genetic technologies to cancer diagnostics and their application to the practice of pathology. We have overcome numerous hurdles to develop high-throughput technologies for rapid and efficient genetic analysis of tumor samples from cancer patients. These tools have changed cancer diagnostics at the Massachusetts General Hospital (MGH) and have been adopted by other cancer centers, both nationally and internationally. We are also exploring the development of predictive biomarkers for lung, breast, and brain cancer and developing new tools to better understand the role of copy number variants (CNVs) and gene fusions in human disease.

Iafrate Laboratory

Aymen Baig
Darrell Borger, PhD
Ju Cheng, PhD
Dora Dias-Santagata, PhD, FACMG
Krista Hu
A. John Iafrate, MD, PhD
Long Le, MD, PhD
Jochen Lennerz, MD, PhD
Maristela Onozato, MD

Our lab has focused efforts on developing highly complex molecular analyses of tumor genetics using novel technologies. We have a strong interest in the clinical implementation of genetic screening technologies that can help direct targeted therapies, focusing on lung, breast and brain tumors. Our recent contributions in the treatment of a subset of non-small cell lung carcinoma (NSCLC) with rearrangements of the ALK tyrosine kinase, and with rearrangements of the ROS1 tyrosine kinase with a small molecule kinase inhibitor (crizotinib), underscore the promise of personalized cancer care. For the phase 1 trial of crizotinib in advanced stage NSCLC, we used a fluorescence in situ hybridization (FISH) assay to screen for ALK rearrangements in archived pathology lung cancer specimens. We screened greater than 1,500 patients to identify the >100 patients who eventually were enrolled into the "expanded cohort" of ALK-positive patients in the phase 1 trial. The observed 65% response rate and 10-month progression-free survival, resulted in rapid FDA approval of both crizotinib and the companion FISH diagnostic. More recently we have focused on the clinicopathologic analysis, and therapeutic approaches to MET exon 14 skipping variants in NSCLC.

We have developed and deployed next generation sequencing to detect chromosomal rearrangements in tumor tissue, with on-going studies that assess the relative sensitivity in much larger clinical cohorts. The method we have developed, termed "anchored multiplex PCR" or AMP, is an efficient target enrichment technology, allowing for 100s of targets to be simultaneously analyzed from small tissue samples. We have used AMP to screen thousands of tumor samples, and have uncovered numerous novel driver fusion genes. Our lab is now focused on modeling novel fusions in vitro and developing therapeutic approaches to screening these fusions. AMP is also the basis for novel assays in other areas, including (1) mapping off-target rates for CRISPR-CAS genome editing; (2) sequencing and mapping the distribution of IgH and TCR rearrangements in tumor samples; and (3) ultra-high sensitive mutation calling in circulating tumor cells and cell free plasma samples. We have also initiated studies of tumor heterogeneity;



Multiplex FISH to detect copy number changes in circulating tumor cells.

these efforts focus on gene amplification of receptor tyrosine kinases in glioblastoma. This work has revealed a new subclass of brain tumors with mosaic gene amplification of up to three kinases in distinct but intermingled cell populations within the same tumor, forming a mosaic pattern. We found that each subpopulation was actively proliferating and contributing to tumor growth. Detailed genetic analysis found that different subpopulations within a particular tumor shared other gene mutations, indicating that they had originated from the same precursor cells. Mapping the location of different subpopulations in the brain of a glioblastoma patient suggested that each subpopulation may serve a different function in the growth and spread of the tumor. Our lab has developed novel highly-multiplexed FISH technology to address how many genes show copy number heterogeneity, and to study the spatial distribution of such populations. We are exploring the therapeutic implications of such driver gene heterogeneity in cell line model systems of glioblastoma using genome-wide CRISPR knock out screens.

Selected Publications:

Matissek KJ, Onozato ML, Sun S, Zheng Z, Schultz A, Lee J, Patel K, Jerevall PL, Saladi SV, Macleay A, Tavallai M, Badovinac-Crnjevic T, Barrios C, Beşe N, Chan A, Chavarri-Guerra Y, Debiasi M, Demirdögen E, Egeli Ü, Gökgöz S, Gomez H, Liedke P, Tasdelen I, Tolunay S, Werutsky G, St Louis J, Horick N, Finkelstein DM, Le LP, Bardia A, Goss PE, Sgroi DC, **Iafrate AJ**, Ellisen LW. Expressed Gene Fusions as Frequent Drivers of Poor Outcomes in Hormone Receptor-Positive Breast Cancer. *Cancer Discov*. 2018 Mar;8(3):336-353.

Heist RS, Shim HS, Gingipally S, Mino-Kenudson M, Le L, Gainor JF, Zheng Z, Aryee M, Xia J, Jia P, Jin H, Zhao Z, Pao W, Engelman JA, and **Iafrate AJ**. MET Exon 14 Skipping in Non-Small Cell Lung Cancer. *Oncologist*. 2016; 21(4):481-486.

Zheng Z, Liebers M, Zhelyazkova B, Cao Y, Panditi D, Chen J, Robinson HE, Chmielecki J, Pao W, Engelman JA, **Iafrate AJ***, Le LP*. Anchored multiplex PCR for targeted next-generation sequencing. *Nat Medicine*. 2014; Nov. 10.

Shaw AT, Ou SH, Bang YJ, Camidge DR, Solomon B, Salgia R, Riely GJ, Varella-Garcia M, Shapiro GI, Costa DB, Doebele RC, Le LP, Zheng Z, Tan W, Stephenson P, Shreeve SM, Tye LM, Christensen JG, Wilner K, Clark JW, **Iafrate AJ**. Crizotinib in ROS1-Rearranged Non-Small Cell Lung Cancer. *N Engl J Med*. 2014; Sept. 27.

Snuderl M, Fazlollahi L, Le LP, Nitta M, Zhelyazkova BH, Davidson CJ, Akhavanfard S, Cahill DP, Aldape KD, Betensky RA, Louis DN, **Iafrate AJ**. Mosaic amplification of multiple receptor tyrosine kinase genes in glioblastoma. *Cancer Cell*. 2011; 20:810-7.

*Co-corresponding authors

Othon Iliopoulos, MD

The Iliopoulos laboratory works on the main mechanisms underlying the reprogramming of cancer cell metabolism and cancer angiogenesis with the goal to develop mechanism-based strategies for selectively killing cancer cells. We use Renal Cell Carcinoma (RCC) as a model disease of altered cancer metabolism and angiogenesis mechanisms. Cancer cells transform their metabolism to adapt to the needs of fast growth and to compete with the surrounding normal cells for nutrients and oxygen. In addition to a reprogrammed metabolism, cancer cells stimulate the growth of new blood vessels that bring blood to them, a phenomenon known for many years as "cancer angiogenesis". The laboratory identifies and validates therapeutic targets that disrupt these processes.

• • •

Iliopoulos Laboratory

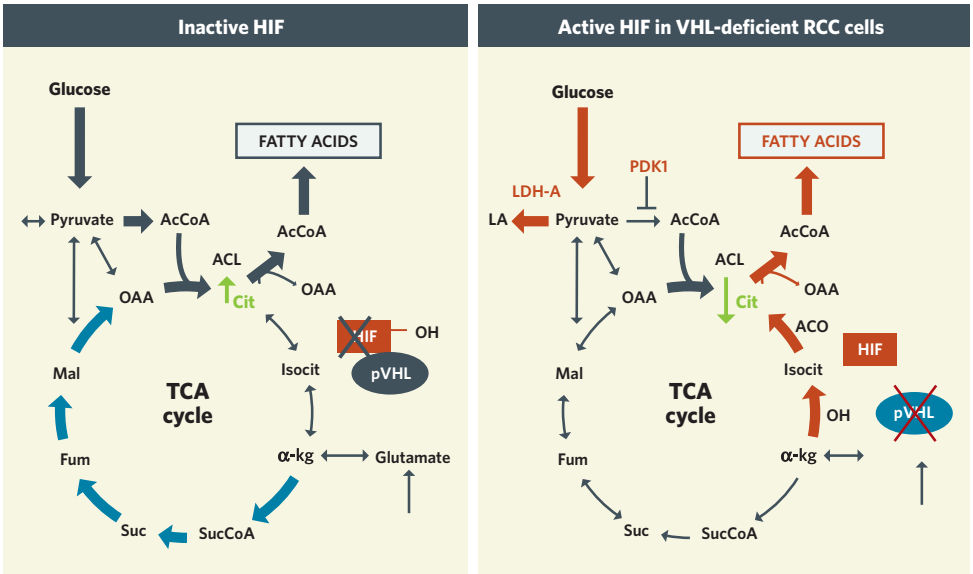
Othon Iliopoulos, MD
Evmorphia Konstantakou, PhD
Ravi Sundaram, BS
Yun Liao

Discovery and validation of therapeutic targets for treatment of renal cell carcinoma

My laboratory uses Renal Cell Carcinoma (RCC) as a disease model to study cancer metabolism and angiogenesis. The overwhelming majority of RCC tumors (more than 90%) lack the VHL tumor suppressor protein. The main function of this protein in the cells is to keep Hypoxia Inducible Factor 2 (HIF2a) under physiologic control; VHL allows the expression of HIF2a only when the cells lack nutrients or the oxygen level drops within a cell (hypoxia). HIF2a is a protein that binds DNA and activates the expression of many genes that interfere with cancer angiogenesis and metabolism. In other words, HIF2a is a "master regulator" of cancer metabolism and angiogenesis. RCCs that lack VHL have continuous expression of HIF2a, independently of the oxygen or nutrient levels within the cell. This inappropriate activation turns HIF2a into an oncogenic "driver" of RCC tumors. In addition to mutations in the VHL gene, mutations in other metabolic enzymes such Succinate Dehydrogenase (SDH) and Fumarate Hydratase (FH) are also linked to the development of RCC. Taken together, these data suggest that the initiation and progression of RCCs depend on metabolic and angiogenic reprogramming. Detailed understanding of the molecular events that regulate cancer angiogenesis and metabolism will lead to rational selection of molecular targets for anticancer drug development.

Discovery and development of hypoxia inducible factor 2a (HIF2a) inhibitors for treatment of renal cell carcinoma and other HIF2a-dependent cancers

We screened libraries of chemical compounds and discovered chemical molecules that significantly and specifically decrease the expression of HIF2a (Zimmer M. et al. *Molecular Cell* 2008; 32(6): 838-48). We used these HIF2a inhibitors as chemical biology probes and discovered that they suppress the expression of HIF2a by activating IRP1. We thus proved a crosstalk between the iron and oxygen sensing mechanisms within the cell. We demonstrated that the HIF2a inhibitors discovered are "active" and that they reverse the consequences of VHL protein loss (Metelo AM. *Journal Clinical Investigation* 2015; 125(5): 1987-97). Our chemical HIF2a inhibitors are very promising agents for treating RCC.



Expression of Hypoxia Inducible Factor HIF2a rewires the central carbon metabolism in renal cell cancer.

Targeting the metabolic reprogramming of RCC and HIF2a expressing tumors; from the lab to the bedside

We used metabolic flux analysis to show that hypoxic cells use glutamine as a carbon source for anabolism. We showed that low oxygen levels or HIF2a expression reprogrammed cells to use glutamine in a "reverse" TCA cycle to produce the metabolites required for anabolic reactions, a process called Reductive Carboxylation. These observations provided insights into a mechanism by which hypoxic and HIF2a expressing cancer cells compensate for the Warburg phenomenon (Metallo et al. *Nature* 2012; 481(7381): 380-4). We delineated the mechanism driving Reductive Carboxylation and proved that reductive carboxylation does not only happen in cultured cells, but can also be detected in human RCC tumors growing as xenografts in mice. We therefore provided for the first time, in vivo evidence for the utilization of glutamine in tumors through reductive carboxylation (Gameiro et al. *Cell Metabolism* 2013; 17(3): 372-385). Recently, we showed that inhibition of Glutaminase 1 (GLS1) decreases significantly the intracellular pyrimidines and results in DNA replication stress in HIF-hypoxia driven cancer cells. Treatment of cancer cells with GLS1 and PARP inhibitors resulted in dramatic suppression of RCC in xenograft models (*J Clin Invest*. 2017; 127(5): 1631-1645).

We brought these fundamental observations of my laboratory on glutamine metabolism to the clinic. We initiated a Phase 1 trial with Glutaminase 1 (GLS1) inhibitors for patients with RCC and triple negative breast cancers nationwide. We are now opening a new clinical trial of GLS1 inhibitor CB-839 and PARP inhibitor combination treatment for patients with RCC, prostate, triple negative and ovarian cancer.

Modeling Renal Cell Carcinoma in the zebrafish

Zebrafish with homozygous inactivating mutations in VHL gene recapitulate aspects of the human VHL disease, including abnormal proliferation of their kidney epithelium. We are using the zebrafish as a model system to model the diverse pathways that lead to renal cell carcinoma development.

Selected Publications:

Okazaki A, Gameiro PA, Christodoulou D, Laviollette L, Schneider M, Chaves F, Stemmer-Rachamimov A, Yazinski SA, Lee R, Stephanopoulos G, Zou L, **Iliopoulos O**. Glutaminase and poly(ADP-ribose) polymerase inhibitors suppress pyrimidine synthesis and VHL-deficient renal cancers. *J Clin Invest*. 2017; 127(5): 1631-1645. (Research Highlights "Targeting metabolism in RCC" in *Nature Reviews Nephrology*. 2017; 13, 320.

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Gameiro PA, Yang J, Metelo AM, Pérez-Carro R, Baker R, Wang Z, Arreola A, Rathmell WK, Olumi A, López-Larrubia P, Stephanopoulos G and **Iliopoulos O**. HIF mediated reductive carboxylation occurs in vivo through regulation of citrate levels and sensitizes VHL-deficient cells to glutamine deprivation. *Cell Metabolism*. 2013;17 (3): 372-385.

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Zimmer M, Ebert BL, Neil C, Brenner K, Papaioannou I, Melas A, Tolliday N, Lamb J, Pantopoulos K, Golub T, **Iliopoulos O**. Small-molecule inhibitors of HIF-2a translation link its 5'UTR iron-responsive element to oxygen sensing. *Molecular Cell*. 2008; 32(6): 838-48.

*Co-corresponding authors

Russell W. Jenkins, MD PhD

Immunotherapy has transformed the treatment of metastatic melanoma and other cancers, allowing a new avenue of therapeutic options and prolonging lives of many patients. Unfortunately, while immunotherapy is highly effective in some patients, it does not work for every patient and there are no available tests to determine whether or not a patient will respond to immunotherapy before treatment begins. To understand why immunotherapy works for some patients and not others, **the Jenkins laboratory** uses sophisticated tools and techniques to study and investigate the complex and dynamic interactions between cancer cells and the immune system. Our solution to this problem involves a specialized 3-dimensional culture of a patient's own tumor enabling researchers to examine interactions between tumor cells and immune cells. The integration of this novel approach with other emerging technologies is helping us navigate the complex landscape of the tumor immune microenvironment and learn which patients will respond to immunotherapy as well as how to effectively treat cancer patients that do not respond immunotherapy alone.

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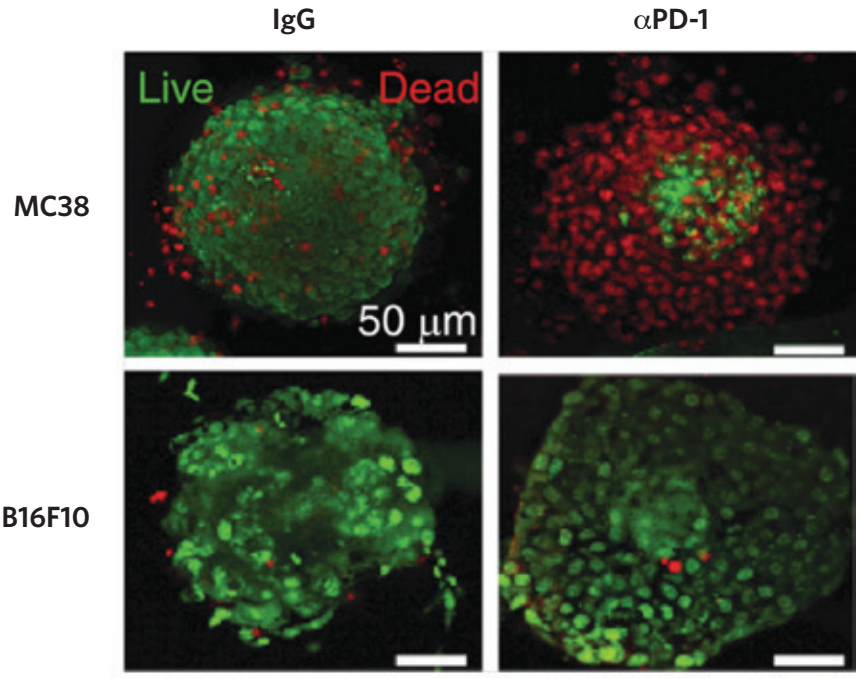
Jenkins Laboratory

(opens Fall 2018)
Jia Gwee, MS
Russell Jenkins, MD, PhD
Greg Rybacki, MS
Yi Sun, PhD

Precision cancer medicine currently focuses on knowledge of the cancer mutation repertoire and the tailored application of drugs that target altered genes or pathways in individual patients, such as use of BRAF inhibitors in patients with BRAF mutant melanoma. Immune checkpoint inhibitors targeting the PD-1/PD-L1 pathway have shown dramatic and durable clinical responses in melanoma and others cancers, but robust predictive biomarkers are lacking and innate resistance is common. Thus, a critical need exists for more sophisticated ex vivo functional testing modalities that recapitulate human tumor biology to predict response to targeted and immune-based therapies and to develop personalized treatment plans in real-time.

Major focus areas of the Jenkins lab include (1) identifying and characterizing mechanisms of response and resistance to PD-1 blockade, (2) discovering novel therapeutic strategies to overcome resistance to PD-1 blockade, and (3) using the MDOTS/PDOTS as a functional precision medicine platform for the development of novel combinations, and ultimately, personalized immunotherapy to tailor immunotherapy treatment to individual patients. Improved understanding of the response to immune checkpoint inhibitors within the tumor microenvironment will facilitate efforts to identify predictive biomarkers/models for immune checkpoint blockade in real-time, as well as future efforts to screen for therapeutic combinations that enhance the response to immune checkpoint blockade, and may ultimately provide a platform for the 'personalization' of immunotherapy.

Our novel approach for evaluating ex vivo response to PD-1 blockade utilizes murine- and patient-derived organotypic tumor spheroids (MDOTS/PDOTS) cultured in a 3-dimensional microfluidic system. Our study which was recently published in *Cancer Discovery* (Jenkins



Live/Dead analysis (Acridine Orange – Green-Live; Propidium Iodide – Red-Dead) of murine-derived organotypic tumor spheroids (MDOTS) from PD-1 sensitive (MC38) and resistant (B16F10) syngeneic mouse models treated ex vivo with IgG or anti-PD-1 (10 μg/mL) for 6 days in 3D microfluidic culture (ref: Jenkins et al. Cancer Discovery 2018).

et al., Cancer Discovery 2018; PMID: 29101162), has shown that organotypic tumor spheroids isolated from fresh mouse and human tumor samples retain autologous lymphoid and myeloid cell populations, including antigen-experienced tumor infiltrating CD4 and CD8 T lymphocytes, and respond to PD-1 blockade in short-term *ex vivo* culture. Furthermore, we have demonstrated that tumor killing was recapitulated *ex vivo* using MDOTS derived from the anti-PD-1 sensitive MC38 syngeneic mouse cancer model, whereas relative resistance to anti-PD-1 therapy was preserved in the CT26 and B16F10 syngeneic models. Our focused evaluation of rational therapeutic combinations to enhance response to PD-1 blockade using *ex vivo* profiling of MDOTS revealed TBK1 inhibition as a novel strategy to enhance sensitivity to PD-1 blockade, which effectively predicted tumor response *in vivo*. Our findings demonstrated the feasibility of *ex vivo* profiling of PD-1 blockade and offer a novel functional approach for the selection of immunotherapeutic combinations. The ultimate goals of these efforts are to identify and characterize novel features of response/resistance to PD-1 blockade and to identify novel therapeutic strategies to overcome resistance to anti-PD-1 therapy, ultimately to bring forward into human clinical trials.

Selected Publications:

Cañadas I, Thummalapalli R, Kim JW, Kitajima S, **Jenkins RW**, et al. Tumor innate immunity primed by specific interferon-stimulated endogenous retroviruses. *Nat Med.* 2018 Aug;24(8):1143-1150..

Li S, Liu S, Deng J, Akbay EA, Hai J, Ambrogio C, Zhang L, Zhou F, **Jenkins RW**, Adeegbe DO, Gao P, Wang X, Paweletz CP, Herter-Sprie GS, Chen T, Gutierrez Quiceno L, Zhang Y, Merlino AA, Quinn MM, Zeng Y, Yu X, Liu Y, Fan L, Aguirre AJ, Barbie DA, Yi X, Wong KK. Assessing Therapeutic Efficacy of MEK Inhibition in a KRAS G12C-Driven Mouse Model of Lung Cancer. *Clin Cancer Res.* 2018 Jun 26. PMID: 29945997

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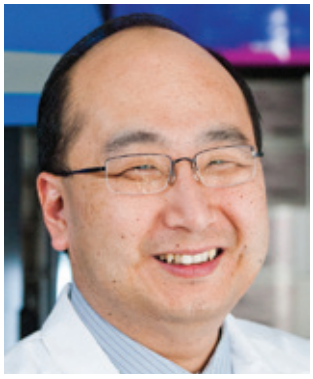
Deng J, Wang ES, **Jenkins RW**, et al. CDK4/6 Inhibition Augments Antitumor Immunity by Enhancing T-cell Activation. *Cancer Discov.* 2018;8(2):216-33. PubMed PMID: 29101163

Kim JW, Abudayyeh OO, Yeerna H, Yeang CH, Stewart M, **Jenkins RW**, Kitajima S, Konieczkowski DJ, Medetgul-Ernar K, Cavazos T, Mah C, Ting S, Van Allen EM, Cohen O, Mcdermott J, Damato E, Aguirre AJ, Liang J, Liberzon A, Alexe G, Doench J, Ghandi M, Vazquez F, Weir BA, Tsherniak A, Subramanian A, Meneses-Cime K, Park J, Clemons P, Garraway LA, Thomas D, Boehm JS, Barbie DA, Hahn WC, Mesirov JP, Tamayo P. Decomposing Oncogenic Transcriptional Signatures to Generate Maps of Divergent Cellular States. *Cell Syst.* 2017 Aug 23;5(2):105-118.e9. PMID:28837809

Yang S, Imamura Y, **Jenkins RW**, Cañadas I, Kitajima S, Aref A, Brannon A, Oki E, Castoreno A, Zhu Z, Thai T, Reibel J, Qian Z, Ogino S, Wong KK, Baba H, Kimmelman AC, Pasca Di Magliano M, Barbie DA. Autophagy Inhibition Dysregulates TBK1 Signaling and Promotes Pancreatic Inflammation. *Cancer Immunol Res.* 2016 Jun;4(6):520-30.



J. Keith Joung, MD, PhD

The Joung laboratory is developing strategies to reprogram the genomes and epigenomes of living cells to better understand biology and treat disease. We have developed and continue to optimize molecular tools for customized genome editing including engineered zinc finger, transcription activator-like effector (TALE), and RNA-guided CRISPR-Cas-based systems. These platforms enable scientists to alter the DNA sequence of a living cell—from fruit flies to humans—with great precision. These technologies are based on designer DNA-binding and RNA-guided proteins engineered to recognize and cleave specific genomic sequences. We also use these targeting methodologies to direct various other regulatory elements to enable activation, repression, or alteration of histone modifications of specific genes. These tools have many potential uses in cancer research and may lead to more efficient gene therapy capable of correcting disease-related mutations in human cells.

Joung Laboratory

James Angstman
Maggie Bobbin, PhD
Peter Cabeceiras
Rebecca Cottman
Stacy Francis
Julian Grunewald, MD
Joy Horng
Jonathan Hsu
Kurt Ibrahim
J. Keith Joung, MD, PhD
Jay Jun
Daniel Kim
Vikram Pattanayak, MD, PhD
Nicholas Perry
Karl Petri, MD
Kanae Sasaki
Alexander Sousa
Esther Tak, PhD

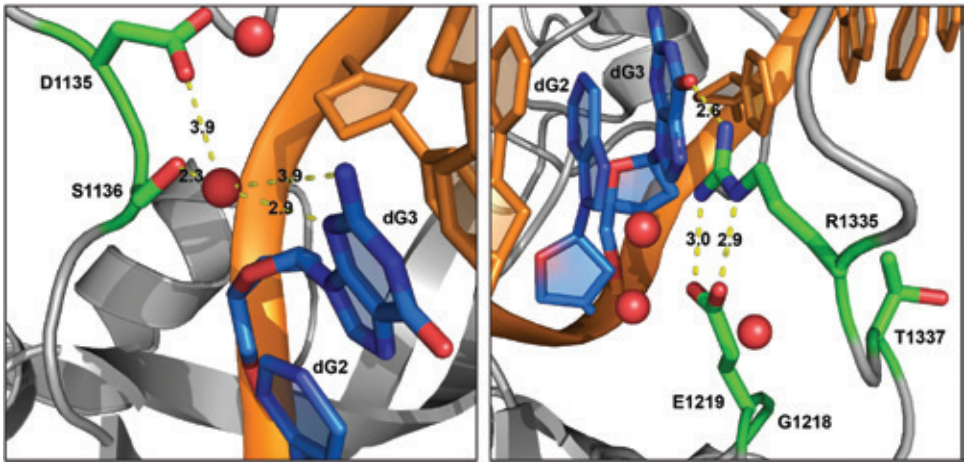
The Joung Laboratory develops technologies for genome and epigenome editing of living cells and organisms using engineered zinc finger, transcription activator-like effector (TALE), and RNA-guided CRISPR-Cas9-based systems and explores their applications for biological research and gene therapy.

Genome Editing Using Targeted Nucleases and Base Editors

Genome editing technology using CRISPR-Cas nucleases was named "Breakthrough of the Year" for 2015 by *Science* magazine. We and our collaborators were the first to demonstrate that these nucleases can function *in vivo* (Hwang & Fu et al., *Nat Biotechnol*. 2013) to modify endogenous genes in zebrafish embryos and the first to show that they can induce significant off-target mutations in human cells (Fu et al., *Nat Biotechnol*. 2013). We have led the field in development of unbiased, genome-wide strategies for profiling the specificities of CRISPR-Cas nucleases including the widely used cell-based GUIDE-seq method (Tsai et al., *Nat Biotechnol*. 2015) and the *in vitro* CIRCLE-seq method (Tsai et al., *Nat Biotechnol*. 2017). We have recently shown that CIRCLE-seq can be used to identify Cas9-induced off-targets *in vivo* (Akcakaya & Bobbin et al., *Nature*, in press). In addition, we have engineered "high-fidelity" Cas9 variants (Kleinstiver & Pattanayak et al., *Nature* 2016) and Cas9 variants with novel DNA binding specificities (Kleinstiver et al., *Nature* 2015; Kleinstiver et al., *Nat Biotechnol*. 2015). More recently, we have developed a novel base editor architecture that shows improved precision and reduced off-target effects (Gehrke et al., *Nat Biotechnol*. 2018).

Epigenome Editing Using Targeted Transcription Factors

We have also performed work showing that the Transcription Activator-Like Effector (TALE) and CRISPR-Cas platforms can also be utilized to create artificial transcription factors that can robustly alter expression of endogenous human genes (Maeder et



Structural representations of DNA recognition by the CRISPR-Cas9 nuclease.

al., *Nat Methods* 2013a; Maeder et al., *Nat Methods* 2013b). We have also developed fusions of engineered TALE domains with the catalytic domain of the TET1 enzyme, enabling the targeted demethylation of CpGs in human cells (Maeder et al., *Nat Biotechnol*. 2013). More recently, we have shown that the CRISPR-Cpf1(Cas12a) platform can be modified to engineer robust transcriptional activators that can efficiently increase endogenous gene expression in human cells (Tak et al., *Nat Methods* 2017).

Selected Publications:

Akcakaya P, Bobbin ML, Guo JA, Malagon-Lopez J, Clement K, Garcia SP, Fellows MD, Porritt MJ, Firth MA, Carreras A, Baccega T, Seeliger F, Bjursell M, Tsai SQ, Nguyen NT, Nitsch R, Mayr LM, Pinello L, Bohlooly-Y M, Aryee MJ, Maresca M, **Joung JK**. In vivo CRISPR editing with no detectable genome-wide off-target mutations. *Nature* 2018, in press.

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Kleinstiver BP, Pattanayak V, Prew MS, Tsai SQ, Nguyen NT, Zheng Z, **Joung JK**. High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. *Nature*. 2016 Jan 28; 529(7587): 490-5.

Li Lan, MD, PhD

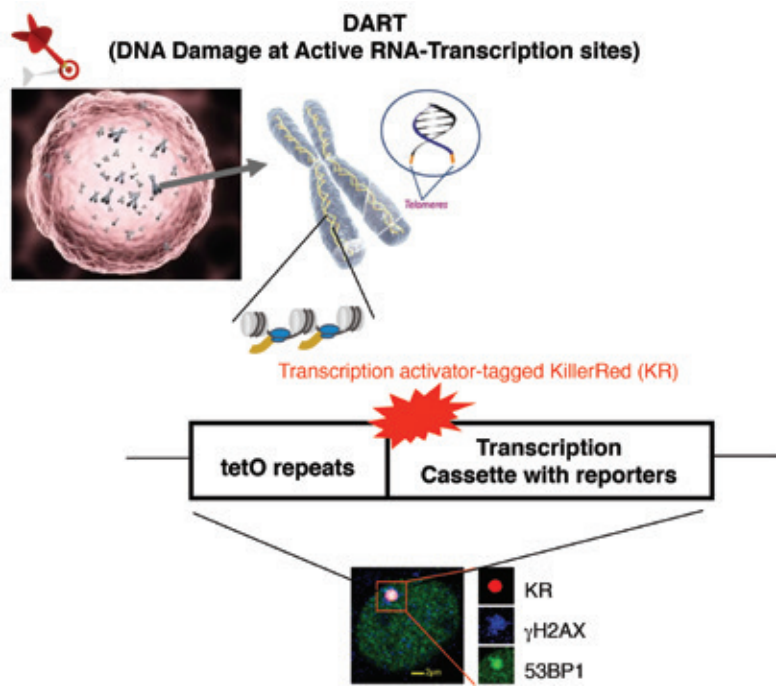
Oxidative DNA damage is a major source of genomic instability during tumorigenesis and aging. The main research interests of **the Lan laboratory** are centered on the mechanisms by which human cells maintain genomic stability against oxidative stress. With a strong appreciation for how human health conditions, especially cancer and neurological maladies, are connected to the loss of genome integrity, ranging from intrinsic genetic predispositions to environmental factors that inflict DNA damage my lab has developed the first single-cell assay to interrogate the molecular mechanisms of oxidative DNA damage response at specific loci in the genome. By combining this innovative assay with state-of-the-art imaging techniques, we have opened new avenues to understanding the oxidative DNA damage response in different chromosomal environments.

Lan Laboratory

Hao Chen, MD
Li Lan, MD, PhD
Junsea Tan, MD
Yumin Wang, PhD*
Haibo Yang, PhD

* Undergraduate student

The ongoing research of my lab is focused on transcription-coupled oxidative DNA damage response and cancer. A growing body of evidence suggests that oxidative stress plays an important role in tumorigenesis, aging, and neurodegenerative diseases. Oxidative stress caused by environmental insults and endogenous metabolites induces DNA base modifications and strand breaks. DNA strand breaks have detrimental effects not only on actively proliferating cells, but also on slowly proliferating cells and terminally differentiated cells. At active transcription sites, RNA Polymerase II can bypass DNA base modifications, but not strand breaks. Given the heterogeneity of cancer cells in tumors, it is critical to understand how dividing and non-dividing cells respond to oxidative DNA damage. One of the main research interests of the Lan laboratory is to understand how oxidative DNA damage response is differentially regulated in transcribed and un-transcribed regions, and in dividing and non-dividing cells. We discovered a novel mRNA-dependent and R loop-mediated homologous recombination (HR) mechanism that specifically promotes repair in the transcribed genome. Thus, our work has revealed an unexpected role for mRNA in HR. Importantly, we show that this mRNA-mediated HR mechanism is able to operate even in G0/G1 cells, challenging the current view that HR only occurs during the S/G2 phase of the cell cycle. Our findings may likely lead to a new paradigm in DNA repair, and to a better understanding of how actively proliferating and slowly proliferating cancer cells respond to oxidative damage. In the near future, we plan to address several important questions on this new pathway that we discovered: (1) Whether and how is the RNA-mediated HR pathway distinct from the canonical HR pathway? (2) How is repair "channeled" into the RNA-mediated HR pathway in transcribed regions? (3) Is the RNA-mediated HR pathway important for tumor suppression? In our ongoing studies, we are exploring the function of RNA modifications in the RNA-mediated HR pathway, and are using advanced super-resolution imaging techniques (STORM and PALM) to study DNA-RNA structural changes at specific sites of DNA damage within the



The Lan laboratory developed the DNA Damage at RNA Transcribed sites (DART) method to precisely introduce oxidative DNA damage at specific transcribed loci in a dose-dependent manner. This is achieved by site-specific positioning of the photo-excitable and ROS-releasing protein KillerRed (KR). This unique method provides a tool to understand how oxidative DNA damage response is differentially regulated in transcribed and un-transcribed regions, and in dividing and non-dividing cells.

genome. We are also using the zebrafish model to assess the functional significance of RNA-mediated HR in vivo. Going forward, we would like to expand our studies to investigate the status of this new RNA-mediated HR repair pathway in cancer cells, its potential function in tumor suppression, and its value as a therapeutic target.

A second research priority of my lab is to understand how telomeres respond to oxidative DNA damage. Telomere dysregulation is a major source of genomic instability and a potential target for cancer therapy. Due to G/C-rich telomeric repeats, telomeres are particularly vulnerable to oxidative stress. Interestingly, telomeres are protected by specific "capping" proteins, making DNA damage response at telomeres significantly different from elsewhere in the genome. More specifically, we are investigating whether and how oxidative damage at telomeres triggers telomere attrition, senescence, and the promotion of tumorigenesis. My lab has established a new method to introduce oxidative damage at telomeres in a highly controlled manner, allowing us, for the first time, to specifically follow the oxidative damage response at telomeres. In several projects, we have investigated how HR factors are regulated by shelterin proteins at telomeres during the oxidative damage response. The recruitment of repair factors to telomeres is coordinately regulated by poly-ADP-ribosylation, phosphorylation, SUMOylation, and ubiquitylation of TRF1 to protect cancer cells from telomere damage. Our future goal is to investigate whether and how the mechanisms orchestrating oxidative damage response at telomeres may contribute to the suppression of tumorigenesis and aging,, and how we can exploit this specific vulnerability of cancer cells in therapy.

Selected Publications:

Teng Y, Yadav T, Duan M, Tan J, Xiang Y, Gao B, Xu J, Liang Z, Liu Y, Nakajima S, Shi Y, Levine AS, Zou L, **Lan L**. ROS-Induced R Loops Trigger a Transcription-Coupled but BRCA1/2-Independent Homologous Recombination Pathway through CSB. *Nature Communications*, in press.

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David M. Langenau, PhD

Most pediatric patients whose sarcoma or leukemia recurs will succumb to their disease. The focus of **the Langenau laboratory** is to uncover the mechanisms that drive progression and relapse in pediatric tumors with the long-term goal of identifying new therapeutic drug targets to treat relapse and refractory disease. One approach we have used is to add drugs to the water of novel zebrafish models of pediatric sarcoma and leukemia that mimic human malignancy. We then imaged tumor growth in the zebrafish and utilize detailed imaging studies to visualize tumor cells in live animals to assess how cellular heterogeneity drives continued tumor growth. Capitalizing on insights gained from our zebrafish models of cancer, we are now extending our findings to human T-cell acute lymphoblastic leukemia and rhabdomyosarcoma.

Langenau Laboratory

Alexandra Bacquelaine Veloso, PhD
Dalton Brunson
Daniel Do
Tiffany Eng, PhD
Elaine Garcia*
Madeline Hayes, PhD
Mathijs Kint
Aleander Jin
David Langenau, PhD
Saara Laukkenen
Karin McCarthy
Alessandra Welker, PhD
Yun Wei, PhD
Chuan Yan, PhD
Qiqi Yang, PhD

* PhD Candidates

Identifying molecular pathways that drive progression and relapse in pediatric cancer...

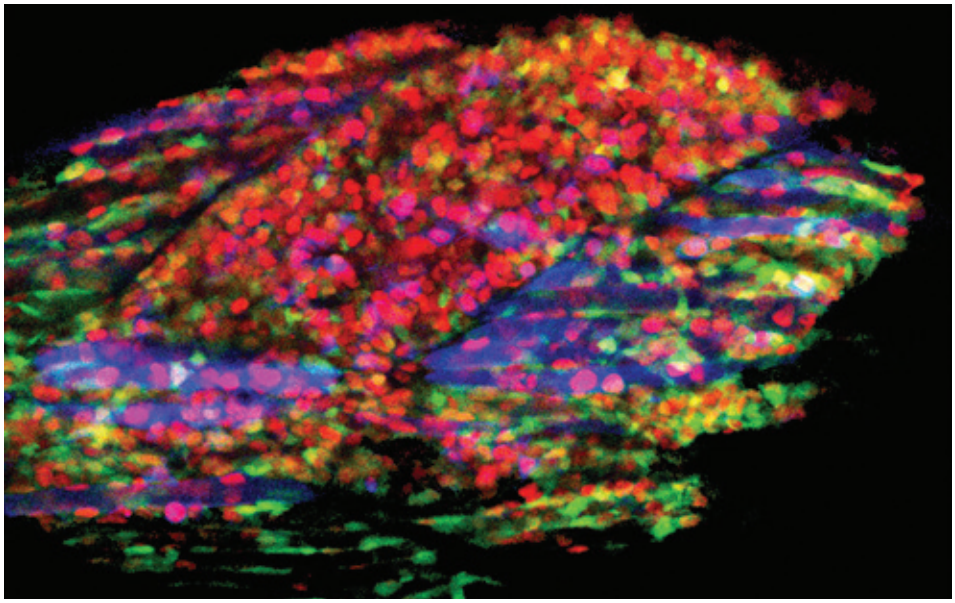
The Langenau laboratory research focus is to uncover relapse mechanisms in pediatric cancer. Utilizing zebrafish models of T-cell acute lymphoblastic leukemia (T-ALL) and embryonal rhabdomyosarcoma (ERMS), we have undertaken chemical and genetic approaches to identify novel modulators of progression, therapy-resistance, and relapse.

Uncovering progression-associated driver mutations in T-cell acute lymphoblastic leukemia

T-ALL is an aggressive malignancy of thymocytes that affects thousands of children and adults in the United States each year. Recent advancements in conventional chemotherapies have improved the five-year survival rate of patients with T-ALL. However, patients with relapse disease are largely unresponsive to additional therapy and have a very poor prognosis. Ultimately, 70% of children and 92% of adults will die of relapse T-ALL, underscoring the clinical imperative for identifying the molecular mechanisms that cause leukemia cells to re-emerge at relapse. Utilizing a novel zebrafish model of relapse T-ALL, large-scale trangenesis platforms, and unbiased bioinformatic approaches, we have uncovered new oncogenic drivers associated with aggression, therapy resistance and relapse. A large subset of these genes exert important roles in regulating human T-ALL proliferation, apoptosis and response to therapy. Discovering novel relapse-driving oncogenic pathways will likely identify new drug targets for the treatment of T-ALL.

Visualizing and killing cancer stem cells in embryonal rhabdomyosarcoma

ERMS is a common soft-tissue sarcoma of childhood and phenotypically recapitulates fetal muscle development arrested at early stages of differentiation. Microarray and cross-species comparisons of zebrafish, mouse and human ERMS uncovered the finding that the RAS pathway is activated in a majority of ERMS. Building on this discovery, our laboratory has developed a transgenic zebrafish model of kRASG12D-induced ERMS



Visualizing cancer stem cells in live zebrafish affected with embryonal rhabdomyosarcoma. GFP expression is confined to the myf5+ ERMS-propagating cells, while differentiated nontumor propagating cells are labeled with a nuclear histone-RFP fusion and membrane associated Cyan.

that mimics the molecular underpinnings of human ERMS. We used fluorescent transgenic zebrafish that label ERMS cell subpopulations based on myogenic factor expression to identify functionally distinct classes of tumor cells contained within the ERMS mass. Specifically, the myf5-GFP+ self-renewing cancer stem cell drives continued tumor growth at relapse and is molecularly similar to a non-transformed, activated muscle satellite cell. Building on the dynamic live cell imaging approaches available in the zebrafish ERMS model, our laboratory has undertaken chemical genetic approaches to identify drugs that kill relapse-associated, self-renewing myf5-GFP+ ERMS cells. We are currently assessing a subset of drugs for their ability to regulate growth of human ERMS cells and mouse xenografts.

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Michael S. Lawrence, PhD

Cancer results from alterations to DNA that lead to the activation of oncogenes or the inactivation of tumor suppressors. **The Lawrence laboratory** focuses on understanding the many ways this can happen, using computation as a powerful microscope to study the processes of DNA damage and repair, gene expression and genome replication, and cancer driver genes. Over our lifetimes, DNA slowly accumulates mutations due to environmental toxins and radiation, as well as from naturally occurring copying errors. The vast majority of mutations have little or no effect on a cell, but out of all possible mutations, a few may hit exactly the right place in the genome, where they can act as a "driver mutation," pushing the cell toward aggressive growth and tumor formation. Sequencing the DNA in a tumor reveals not only its driver mutations, but also all the other "passenger mutations" that were present in the tumor-initiating cell. We seek insights about cancer from both driver and passenger mutations.

Lawrence Laboratory

Andrew Dunford, BSc*
Nicholas Haradhvala, BA*
Julian Hess, BS*
Eugene Kwan, PhD
Adam Langenbucher, BS
Michael S. Lawrence, PhD
Rob Morris, PhD
Vishal Thapar, PhD
Jillian Wise, PhD
Ben Wittner, PhD

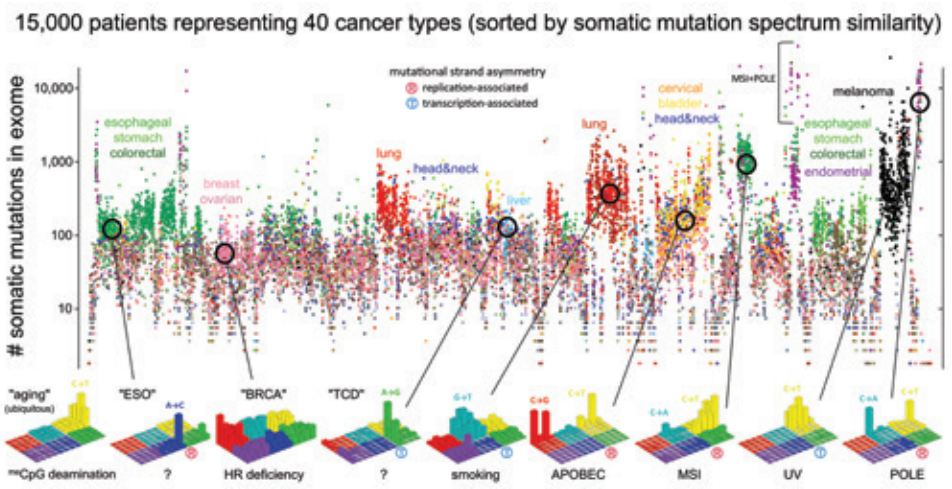
*Associate Computational Biologist based at The Broad Institute

Tumor DNA Sequencing

High-throughput DNA sequencing is a workhorse of biomedical research. There are many challenges in processing the raw DNA sequencing reads from a patient's resected tumor or biopsy material, aligning them accurately to the reference human genome, and then scanning for loci where the tumor DNA differs from the patient's bulk "normal" DNA (e.g. from a blood draw). Distinguishing true somatic mutations from sequencing or alignment artifacts can be tricky, especially for subclonal events present in only a fraction of tumor cells. We are refining a "panel of normals" (PoN) approach, which combats stochastic artifacts seen in the patient's tumor sample, and not in the patient's normal sample but widespread however in many other patients' normal samples. We are continually discovering new artifact modes, making this a highly challenging and unpredictable area of research. Isolating true somatic mutations is crucial for downstream analyses of mutational signatures and driver events.

Analyzing Mutational Signatures

Cancers vary over many orders of magnitude in their total background mutation burden, ranging from very quiet tumor types such as leukemias and childhood tumors, which may have fewer than 10 somatic mutations in their exome, to carcinogen-associated tumor types such as lung cancer and melanoma, which may have over 1000. Mutations have many causes, and each mutagen can leave a telltale signature. For instance, spontaneous deamination of methylated CpG's causes the transition mutations that dominate many tumor types. Mutagens in tobacco smoke cause G-to-T transversions. Ultraviolet radiation causes C-to-T at dipyrimidines. Agitated APOBEC enzymes cause mutations at C's preceded by T. Loss of mismatch repair causes microsatellite instability (MSI), marked by expansion and contraction of simple-sequence repeats, as well as characteristic types of single-base changes. Tumors carrying mutations in the proofreading exonuclease domain of polymerase epsilon (POLE) tend to



Survey of total mutational burden and mutational spectra across many tumor types. Each dot represents a cancer patient whose tumor was subjected to whole-exome DNA sequencing. Vertical position indicates the total number of somatic coding mutations in each patient. Patients are sorted by the similarity of their somatic mutation spectra (i.e. clustering dendrogram order). Colors indicate tissue types (red=lung, orange=cervical, etc.). Lower panel shows nine common mutation spectra with lines drawn to representative sample cohorts. These "Lego plots" indicate relative frequencies of the 96 possible trinucleotide-based mutation types.

accrue C-to-A mutations at the trinucleotide TCT. Very rare "MSI+POLE" cancers show the highest yet known somatic mutation burdens, with upwards of 10,000 coding mutations per patient. Patients affected by MSI and/or POLE mutagenesis are known to experience better clinical outcomes, probably thanks to their high neoantigen loads which attract a powerful immune response. Our most recent research has focused on a less well-studied signal in somatic mutation datasets, mutational asymmetries between the two DNA strands. These illuminate transcriptional or "T-class" mutational patterns, associated with exposure to tobacco smoke, UV radiation, and a yet-unknown agent in liver cancer, as well as replicative or "R-class" patterns, associated with MSI, APOBEC, POLE, and a yet-unknown agent in esophageal cancer.

Tumor Evolution and Drug Resistance

When cancer is treated with therapies that target specific driver mechanisms, the selective pressure on the cancer cells often results in the rapid emergence of drug resistance. For example, lung cancer in non-smokers is often driven by gene fusions of a kinase such as ALK. An initial biopsy may reveal that the patient's tumor is made up entirely of cells with an ALK fusion, leading the oncologist to treat the patient with an ALK inhibitor such as crizotinib. This leads to rapid improvement of the patient, with the tumor shrinking dramatically. However, after a period of treatment (sometimes as short as a few months), the cancer becomes resistant to the inhibitor and the tumor grows back. A repeat biopsy (or sampling of pleural effusions) often reveals a new point mutation in ALK, which in up to a third of patients is exactly the same mutation, L1196M, called the "gatekeeper mutation" because of its ability to block drug binding. By analyzing sequential cancer samples from the patient, we track the emergence of resistant clones and learn how cancer evolves in response to the patient's treatments. Our goal is to understand common mechanisms of drug resistance and how to thwart them.

Selected Publications:

Yoda S, Lin JJ, **Lawrence MS**, Burke BJ, Friboulet L, Langenbucher A, Dardaei L, Prutisto-Chang K, Dagogo-Jack I, Timofeevski S, Hubbeling H, Gainor JF, Ferris LA, Riley AK, Kattermann KE, Timonina D, Heist RS, Iafrate AJ, Benes CH, Lennerz JK, Mino-Kenudson M, Engelman JA, Johnson TW, Hata AN, Shaw AT. Sequential ALK Inhibitors Can Select for Lorlatinib-Resistant Compound ALK Mutations in ALK-Positive Lung Cancer. *Cancer Discov.* 2018 Jun;8(6):714-729.

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Shyamala Maheswaran, PhD

Metastasis, the leading cause of cancer-related deaths, is governed by multiple steps, which are not well understood. Using cell culture and mouse models, as well as patient-derived tumor tissues and tumor cells circulating in the blood (Circulating Tumor Cells/CTCs), **the Maheswaran laboratory** has uncovered novel tumor cell characteristics that promote metastasis in breast cancer patients. Our findings show that cancer cells exist in multiple cellular states, each state exhibiting different characteristics. As such, each breast cancer patient harbors a mixture of tumor cells with different functional properties. We intend to define the functional and molecular properties of these different subclasses of tumor cells and their contribution to metastasis, tumor evolution and drug sensitivity using appropriate experimental models and patient-derived samples. These findings will provide insight into the contribution of these different cancer cell populations to metastasis and their significance as biomarkers and therapeutic targets.

Maheswaran Laboratory*

Brian Chirn
Christina Costantino
Valentine Comaills, PhD
Taronish Dubash, PhD
Richard Ebright**
Hongshan Guo, PhD
Xin Hong, PhD
Elad Horwitz, PhD
Mark Kalinich**
Satoru Matsuda, MD**
Shyamala Maheswaran, PhD
Douglas Micalizzi, MD
Stefanie Morgan, PhD*
Benjamin Nicholson
Britanny Reeves
Tanya Todorova, PhD
Benjamin Wesley
Devon Wiley
Ben Wittner, PhD

* Co-directed with Daniel Haber, MD, PhD

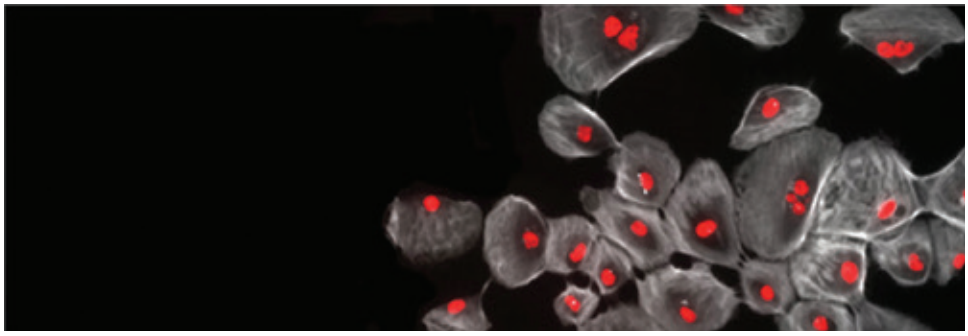
** Graduate students

Mechanisms of Breast Cancer Metastasis

The research in my laboratory is focused on defining the molecular mechanisms that drive breast cancer progression and metastasis. Cancer, initially confined to the primary site, eventually spreads to distal sites, including lung, liver, bone and brain, by invading into the bloodstream. Upon reaching these distal sites, the tumor cells continue to grow and evolve well after removal of the primary tumor resulting in overt metastasis and disease recurrence, the leading causes of cancer-related deaths. Using cell culture and mouse models and patient derived tissues and circulating tumor cells (CTCs) enriched from the blood of women with breast cancer, we characterize the contribution of oncogenic and tumor microenvironment-derived signals, epithelial to mesenchymal transition and tumor heterogeneity to cancer progression and therapeutic responses.

Metastasis through the Prism of Circulating Tumor Cells

I am also collaborating with Drs. Daniel Haber and Mehmet Toner to define cancer biology across several tumor types including breast, prostate, liver and lung cancers as well as melanoma using CTCs isolated from the blood of cancer patients. CTCs represent an extremely rare population of cells in the blood and their isolation presents a tremendous technical challenge. The CTC-iChip developed in Dr. Toner's laboratory enables enrichment of live CTCs through selective removal of blood components; red and white blood cells as well as platelets. Characterizing CTCs has far-reaching implications for both clinical care and defining cancer biology. They enable real time monitoring of tumor cells during disease progression and therapeutic responses, and could possibly be used for early detection of disease. Viable CTCs cultured from patients



Persistent proliferation of cancer cells during epithelial to mesenchymal transition leads to cytokinesis failure resulting in binucleated cells and chromosome missegregation.

provide tremendous insight into the molecular heterogeneity and cellular plasticity of tumors that govern differential biological characteristics and responses to therapy. Characterization of CTCs ties in well with the overall goal of the lab to study cancer metastasis.

Selected Publications:

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*Co-corresponding authors

Marcela V. Maus, MD, PhD

Using the immune system as a cancer treatment has the potential to induce long-term, durable remissions, and perhaps even cures for some patients. The T cells of the immune system are able to specifically kill the target cells they recognize. T cells are also able to persist in the body for many years, and form immune 'memory,' which enables the possibility of long-term protection. **The Maus laboratory** is interested in using genetic engineering techniques to re-direct T cells to find and kill tumor cells, while sparing healthy tissues. We aim to develop new ways to design molecular receptors to target T cells to liquid and solid tumors; use T cells as delivery vehicles for other drugs, and use drugs to help T cells work against tumors; and understand how T cells can work as "living drugs" to treat patients with cancer.

Maus Laboratory

Stephanie Bailey, PhD
Angela Boroughs*
Amanda Bouffard
Ana Castano, MD
Alena Chekmasova, PhD
Bryan Choi, MD, PhD
Matthew Frigault, MD
Kathleen Gallagher, PhD
Max Jan, MD, PhD
Rebecca Larson*
Mark Leick, MD
Leah Marsh
Marcela V. Maus, MD, PhD
Alex Pourzia, **
Lauren Riley
Maria Cabral Rodriguez
Irene Scarfo, PhD
Andrea Schmidts, MD
Xiaoling Yu**

*PhD Candidate

**MD candidate

Immune therapies that engage T cells have the potential to induce long-term durable remissions of cancer. In hematologic malignancies, allogeneic hematopoietic stem cell transplant can be curative, in part due to T-cell mediated anti-tumor immunity, in solid tumors, checkpoint blockades with anti-CTLA-4 or anti-PD-1 monoclonal antibodies can mediate long-term responses by releasing T cells from tightly controlled peripheral tolerance. Chimeric antigen receptors (CARs) are synthetic molecules designed to re-direct T cells to specific antigens. Re-directing T cells with CARs is an alternative method of overcoming tolerance, and has shown great promise in the clinical setting for T cell malignancies such as adult and pediatric acute lymphoblastic leukemia (ALL). This therapy is so effective in ALL that based on relatively small numbers of patients, multiple academic centers and their industry partners have received Breakthrough Designation from the FDA to commercialize CAR T cell products. However, successful application of this form of therapy to other cancers is likely to require refinements in the molecular and clinical technologies.

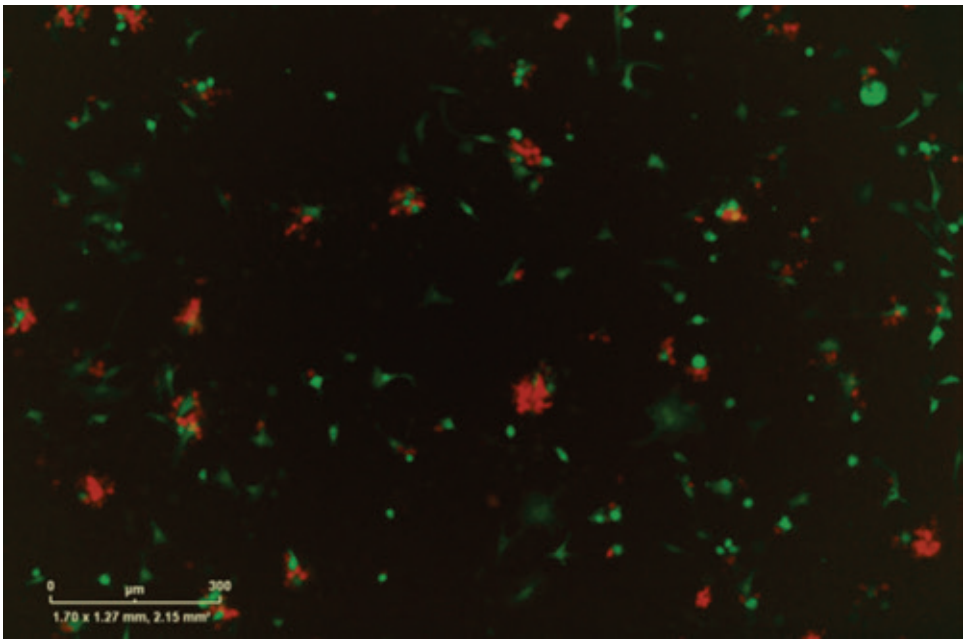
The goal of the Maus lab is to design and evaluate next generation genetically-modified (CAR) T cells as immunotherapy in patients with cancer.

Specifically, next generation T cells that the Maus lab intends to develop includes CAR-T cells that:

1. **Contain molecular improvements in receptor design to enhance specificity, potency, and safety.**

   Most chimeric antigen receptors used to re-direct T cells to a new target are based on enforcing expression of either murine single-chain antibody fragments, natural ligands, or natural T cell receptors. However, novel types of antigen receptors are in development and could be exploited to re-direct T cells such that they can distinguish between antigen expressed on the tumor and the same antigen expressed in healthy tissues. In liquid tumors, it will also be important to improve the safety of CAR T cells, while in solid tumors, the focus is on increasing their potency.

2. **Are administered in combination with other drugs delivered either (a)**



CAR T cells (co-expressing the red fluorescent marker mCherry) surrounding and killing glioblastoma cells (expressing green fluorescent protein).

systemically or (b) as payloads attached to T cells to sensitize tumors to T cell mediated killing and/or potentiate T cell function.

Some recently developed targeted therapies have effects on T cells or tumor cells that potentiates the tumor-killing effects. Alternatively, T cells can be chemically or genetically loaded with drugs to potentiate T cell function, such as cytokines or antibodies to checkpoint inhibitors. In this case, re-directed T cells could be used as a delivery mechanism to target an otherwise toxic drug specifically to the tumor.

3. **Have additional modifications that make CAR T cells (a) resistant to inhibitory mechanisms, (b) imageable, or (c) more feasible to manufacture and administer.**

   Control of T cell function is a complex process orchestrated by a variety of molecules, some of which deliver inhibitory signals. Tumors often express ligands to inhibit T cell function. Using a single vector, genetically modified T cells can be re-directed not only to recognize a new antigen on tumor cells, but also to be resistant to the inhibitory tumor micro-environment.

4. **We aim to understand the basic biology and mechanisms that drive engineered T cell function.**

The MGH Cellular Immunotherapy Program directed by Dr. Maus aims to generate a pipeline of genetically engineered CAR T cells to use as "living drugs" in patients with cancer. The program is composed of a "research and discovery" arm, "a regulatory/translational" arm to be able to test genetically-modified T cells in human subjects (directed by Dr. Alena Chekmasova), and a "clinical/ correlative" sciences arm of immune profiling to examine the engraftment, persistence, and bioactivity of T cell products infused into patients (directed by Dr. Kathleen Gallagher).

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Andrea I. McClatchey, PhD

The McClatchey laboratory focuses on understanding how cells organize their outer membrane or cortex, which, in turn, determines their identity, behavior, and interface with the extracellular environment. Cancer cells exhibit defective membrane organization and therefore interact inappropriately with other cells and with their environment. Our research stems from a longstanding interest in understanding the molecular basis of neurofibromatosis type 2 (NF2), a familial cancer syndrome that is caused by mutation of the *NF2* tumor suppressor gene. The *NF2*-encoded protein, Merlin, and closely related ERM proteins (Ezrin, Radixin, and Moesin), are key architects of the cell cortex.

• • •

McClatchey Laboratory

Christine Chiasson-MacKenzie, PhD

Ching-Hui Liu

Andrea I. McClatchey, PhD

Evan O'Loughlin

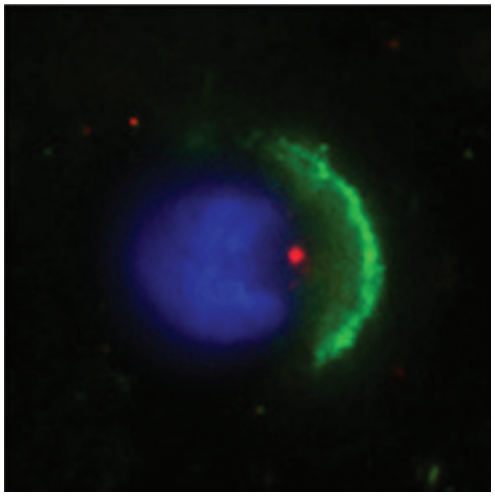
*Graduate student

Understanding morphogenesis and tumorigenesis

The vast array of forms and functions exhibited by different cell types is made possible by the organization of specialized domains within the cell cortex, such as cell:cell and cell:matrix adhesions, the intestinal brush border, neuronal growth cone and immunological synapse. The assembly of such cortical domains involves the coordination of processes occurring at the plasma membrane with those in the underlying cytoskeleton. Central to this coordination is the formation of protein complexes at the plasma membrane that position membrane receptors, control their abundance and activity, and link them to the cortical cytoskeleton, thereby serving both regulatory and architectural functions. The overarching goal of my laboratory is to understand how the organization of protein complexes at the cell cortex contributes to morphogenesis and tumorigenesis. This interest stems from a longstanding dedication to elucidating the molecular basis of neurofibromatosis type 2 (NF2), a familial cancer syndrome that is caused by mutation of the *NF2* tumor suppressor gene.

The *NF2*-encoded protein Merlin is closely related to the ERM proteins (Ezrin, Radixin and Moesin) that link membrane proteins to the cortical cytoskeleton, thereby both stabilizing membrane complexes and stiffening the cell cortex. The proximal goal of our work is to delineate the molecular function of Merlin and identify therapeutic targets for NF2; our work also directly addresses fundamental aspects of basic and cancer cell biology.

Through the generation and analysis of mouse and three dimensional tissue culture models, we identified critical roles for Merlin and the ERM proteins in morphogenesis, homeostasis and tumorigenesis in many tissues including the liver, kidney, intestine, skin and mammary gland. Molecular and cell-based studies suggest that these phenotypes are caused by defective organization of the cortical cytoskeleton, which leads to altered distribution of membrane receptors such as EGFR/ErbBs, cell junction components, and/or protein complexes that guide the orientation and function of the mitotic spindle. We also discovered that a fundamental function of Merlin is to restrict the distribution of Ezrin at the cell cortex and that loss of this activity



The membrane-cytoskeleton linking protein Ezrin forms a cortical 'cap' (green) that instructively positions the centrosome (red) and eventually guides mitotic spindle orientation in dividing cells (the nucleus is stained blue).

underlies several of these phenotypes. In the absence of Merlin, unrestricted cortical Ezrin drives aberrant mechanical stress on cell-cell junctions, altered endocytic trafficking of membrane receptors and abnormal centrosome-to-cortex communication, yielding defective spindle orientation and integrity. These studies provided novel insight into how the organization of the cell cortex governs the identity and behavior of individual cell types and how defective cortical organization contributes to unscheduled cell proliferation, invasion and tumor development.

Ongoing studies extend both basic and translational implications of this work. We have uncovered new mechanistic insight into how Merlin/ERMs organize the biochemical and physical properties of the cell cortex and how this, in turn, controls receptor distribution and spindle orientation/integrity. Importantly, we are also pursuing novel translational avenues that stem directly from our basic studies by delineating the role of unregulated ErbB signaling and aberrant centrosome/spindle function in *NF2*-mutant tumors. Indeed, we have found that *NF2*-mutant tumor cells exhibit altered ErbB trafficking and centrosome/spindle integrity and sensitivity to drugs that target them. We believe that the continued partnering of discovery-based science and translational studies will not only lead to novel therapeutic options for *NF2*-mutant tumors but also advance our understanding of these basic cellular activities that are also known to contribute to other human cancers.

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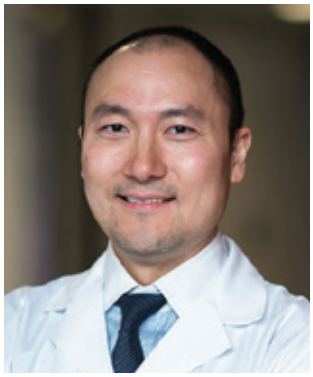


David T. Miyamoto, MD, PhD

The Miyamoto laboratory focuses on the discovery and development of novel biomarkers to guide the personalized treatment of patients with prostate and bladder cancer. We focus on two general classes of biomarkers, namely those based on the molecular profiles of tumor biopsies, and those based on circulating tumors cells (CTCs) in the blood that can be sampled non-invasively and repeatedly. By analyzing these patient-derived specimens, we have identified new molecular predictors of response to therapy and potential mechanisms of treatment resistance. Our overall aim is to develop tools for "real-time precision medicine" to probe the molecular signatures of cancers as they evolve over time, and to guide the precise and rational selection of appropriate therapies for each individual patient with prostate or bladder cancer.

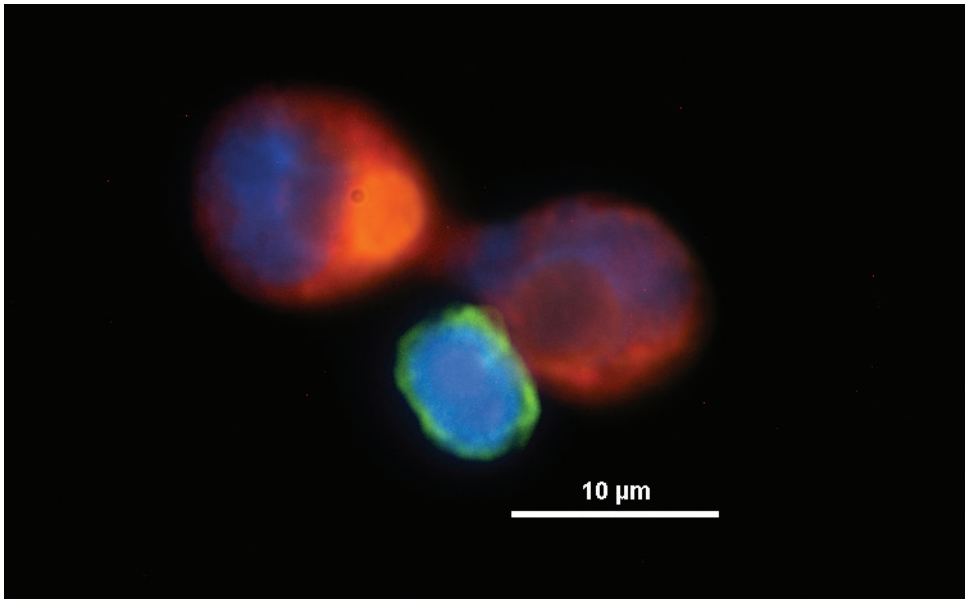
• • •

Miyamoto Laboratory

Katherine Broderick, RN
Rebecca Fisher, BA
William Hwang, MD, PhD
David Miyamoto, MD, PhD
Haley Pleskow, BA

The mission of our translational research laboratory is to develop biomarkers that inform clinical decisions in the management of patients with genitourinary malignancies. In prostate cancer, there is a critical unmet need for predictive biomarkers to guide therapy in settings ranging from localized to metastatic disease. For example, multiple therapeutic options are now available for metastatic castration-resistant prostate cancer (mCRPC) including androgen receptor (AR) targeted therapies, cytotoxic chemotherapy, and PARP inhibitors, but we lack non-invasive biomarkers that can reliably predict treatment responses and guide selection of the most appropriate therapy for each individual patient. In localized prostate cancer, there is an unmet need for molecular biomarkers to guide the rational selection of appropriate management options, which include active surveillance, radical prostatectomy, or radiation therapy with or without androgen deprivation therapy. Similarly, in bladder cancer, muscle-invasive bladder cancer can be treated with either radical cystectomy or bladder-sparing trimodality therapy (transurethral tumor resection followed by chemoradiation), but this decision is often made based on patient or physician preference. For each of these cases, there is an urgent need for biomarkers to guide patients towards the most appropriate therapy based on the biology of the tumor. Our laboratory is centered on discovering and developing such molecular biomarkers.

A major focus of our laboratory is the investigation of circulating biomarkers in cancer patients. Circulating tumors cells (CTCs) are rare cancer cells shed from primary and metastatic tumors into the peripheral blood, and represent a "liquid biopsy" that may be performed repeatedly and non-invasively to monitor treatment efficacy and study tumor evolution during therapy. In collaboration with a multidisciplinary team of bioengineers, molecular biologists, and clinicians at MGH, we have studied the application of novel microfluidic technologies to isolate and analyze CTCs from the blood of cancer patients. We have used CTC analyses to monitor therapy in patients with localized and metastatic prostate cancer, interrogate androgen receptor (AR)



A dividing circulating tumor cell isolated from a prostate cancer patient, immunostained for PSA (red), PSMA (orange), and DNA (blue), adjacent to a leukocyte immunostained for CD45 (green) and DNA (blue).

signaling status in patients undergoing treatment with AR-targeted therapies, and studied the significance of CTC clusters in the development of metastases. Through comprehensive single cell RNA-sequencing of prostate CTCs, we demonstrated that noncanonical Wnt signaling contributes to therapeutic resistance in mCRPC. Recently, we derived RNA signatures in CTCs that predict resistance to AR-targeted therapy in metastatic cancer and early dissemination of disease in localized cancer. Ongoing projects include the development of CTC molecular signatures to predict outcomes after PARP inhibitor therapy in mCRPC, after local therapy in localized prostate cancer, and after bladder-sparing trimodality therapy in patients with muscle-invasive bladder cancer.

A second focus of the laboratory is the development of novel tissue-based biomarkers. We utilize technologies including microfluidic real-time PCR, next-generation sequencing, and branched chain RNA in situ hybridization (RNA-ISH) to evaluate molecular signatures in limited quantities of formalin-fixed paraffin-embedded (FFPE) tumor biopsy tissues. These molecular findings are correlated with clinical outcomes to identify novel biomarkers predictive of treatment response. We developed an RNA-ISH assay to detect the constitutively active androgen receptor mRNA splice variant AR-V7 in archival FFPE tissues, and demonstrated its prognostic value in patients with mCRPC. We recently identified immune and stromal molecular signatures predictive of outcomes after bladder-sparing chemoradiation therapy for bladder cancer. We are currently evaluating these and other candidate biomarkers as predictors of treatment response in prospective clinical trials and carefully defined retrospective clinical cohorts. Through these approaches, we aim to develop circulating and tissue-based biomarkers in a variety of clinical contexts, and develop and actualize the concept "real-time precision medicine", integrating genomic analyses of liquid and tissue biopsies to guide the personalized care of patients with genitourinary malignancies.

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* Co-first authors

Raul Mostoslavsky, MD, PhD

Research in **the Mostoslavsky laboratory** focuses on the crosstalk between chromatin dynamics and cellular metabolism. In particular, we have focused on sirtuins, a family of proteins first discovered in yeast that plays a critical role in many human diseases, including cancer. The yeast protein Sir2 enables yeast cells to survive under conditions of nutrient stress and functions as a modulator of lifespan. While recent studies indicate that some of the mammalian sirtuin (SIRT) homologues also play a role in stress resistance and metabolic homeostasis, their precise molecular functions remain unclear. Most of our work involves the Sir2 mammalian homolog known as SIRT6. Our research indicates that SIRT6 modulates glucose metabolism and DNA repair and functions as a strong tumor suppressor gene. Using transgenic mouse models and other experimental systems, we are exploring the role of SIRT6 and metabolism in tumorigenesis and other disease processes, as well as trying to understand the crosstalk between metabolism and epigenetics.

Mostoslavsky Laboratory

Ittai Ben Porath**
Ruben Boon, PhD
Jee-Eun Choi, PhD
Jean-Pierre Etchegaray, PhD
Christina Ferrer, PhD
Yasmin Hernandez Barco, MD
Sam Linder*
Raul Mostoslavsky, MD, PhD
Lara Roach*
Giorgia Silveira, PhD
Nicole Smith, BSc
Jordan Todd*

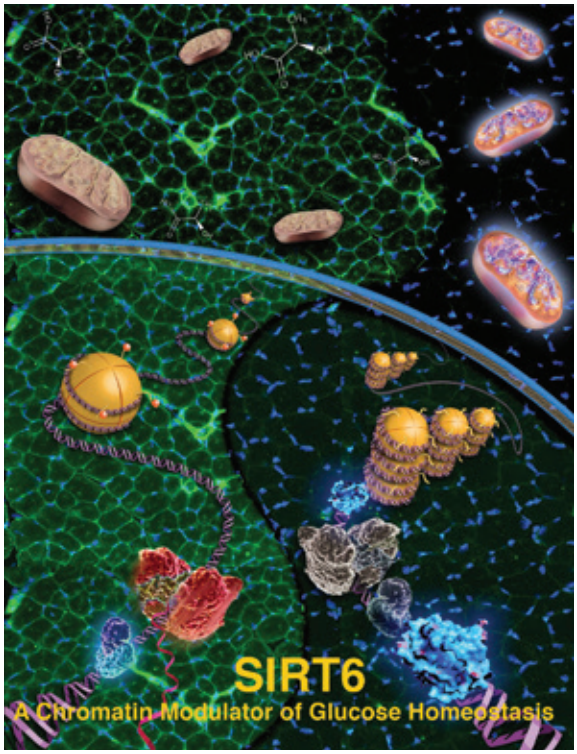
* Graduate student

** Visiting Professor, The Hebrew University Jerusalem

The DNA and the histones are arranged in the nucleus in a highly condensed structure known as chromatin. Cellular processes that unwind the double helix—such as transcription, replication and DNA repair—have to overcome this natural barrier to DNA accessibility.

Multicellular organisms also need to control their use of cellular energy stores. Glucose metabolism plays a crucial role in organismal homeostasis, influencing energy consumption, cell proliferation, stress resistance and lifespan. Defective glucose utilization causes numerous diseases ranging from diabetes to an increased tendency to develop tumors. For cells to respond appropriately to changes in energy status, they need a fine-tune system to modulate chromatin dynamics in order to respond to metabolic cues. Reciprocally, chromatin changes necessary for cellular functions need as well to be coupled to metabolic adaptations.

Our lab is interested in understanding the influence of chromatin on nuclear processes (gene transcription, DNA recombination and DNA repair) and the relationship between chromatin dynamics and the metabolic adaptation of cells. One of our interests is on the study of a group of proteins called SIRTs, the mammalian homologues of the yeast Sir2. Sir2 is a chromatin silencer that functions as an NAD-dependent histone deacetylase to inhibit DNA transcription and recombination. In the past few years, we have been exploring the crosstalk between epigenetics and metabolism. In particular, our work has focused on the mammalian Sir2 homologue, SIRT6. In recent years, we have identified SIRT6 as a key modulator of metabolism. Mice lacking SIRT6 exhibit severe metabolic defects, including hypoglycemia and hypoinsulinemia. SIRT6 appears to modulate glucose flux inside the cells, functioning as a histone H3K9 deacetylase to silence glycolytic genes acting as a coexpressor of Hif1alpha, in this way directing glucose away from to reduce intracellular ROS levels. This function appears critical for glucose



SIRT6: A Chromatin Modulator of Glucose Homeostasis

homeostasis, as SIRT6 deficient animals die early in life from hypoglycemia. Remarkably, SIRT6 acts as a tumor suppressor in colon cancer, regulating cancer metabolism through mechanisms that by-pass known oncogenic pathways. Cancer cells prefer fermentation (i.e., lactate production) to respiration. Despite being described by biochemist and Nobel laureate Otto Warburg decades ago (i.e., the Warburg effect), the molecular mechanisms behind this metabolic switch remain a mystery. We believe SIRT6 may function as a critical modulator of the Warburg effect, providing a long-sought molecular explanation to this phenomenon. We have also uncovered key roles for SIRT6 in DNA repair (anchoring the chromatin remodeler SNF2H to DNA breaks) and early development (acting as a repressor of pluripotent genes), indicating broad biological functions for this chromatin deacetylase. More recently, we identified SIRT6 as a robust tumor suppressor in pancreatic cancer, where it silences the oncofetal protein Lin28b, protecting against aggressive tumor phenotypes. As such, SIRT6 represents an example of a chromatin factor modulated by cancer cells to acquire "epigenetic plasticity".

Our current studies are directed at determining how the DNA repair and metabolic functions of SIRT6 may be related to each other. We are as well exploring novel metabolic liabilities in cancer, as well as broader chromatin roles in DNA repair. We use a number of experimental systems, including biochemical and biological approaches, as well as genetically engineered mouse models.

Projects:

1. Deciphering how SIRT6 regulates chromatin structure
2. Determining the role of SIRT6 in tumorigenesis using mouse models
3. Elucidating the role of histone modifications and chromatin dynamics in DNA repair
4. Determining molecular crosstalk between epigenetics and metabolism
5. Assessing metabolic liabilities in cancer and metastases

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Ferrer C, Alders M, Postma AV, Park S, Klein MA, Cetinbas M, Pajkrt E, Glas A, van Koningsbruggen S, Christoffels VM, Mannens MMAM, Knelt L, Etchegaray JP, Sadreyev R, Denu JM, **Mostoslavsky G**, van Maarle MC, and Mostoslavsky, R. (2018). An inactivating mutation in the histone deacetylase SIRT6 causes human perinatal lethality. *Genes & Dev*., 2018 32, 373-388.

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Mo Motamedi, PhD

Research in **the Motamedi Laboratory** focuses on a molecular memory system, called epigenetics, which enables cells to establish stable fates during development or resist stress in response to environmental changes. Epigenetic mechanisms give cells new properties often by turning groups of genes on and off at a given time. A focus of the lab is studying the molecular machinery that permits cells to transmit this information to progeny cells upon division. Another focus for the lab is cellular dormancy. Recently, scientists have discovered that a major reason for cancer resistance and recurrence is that a small number of dormant cancer cells originating from the primary tumor disperse throughout the body. These cancer cells are long-lived and can exit dormancy forming tumors years after remission. None of the existing therapies target dormant cancer cells. By studying dormancy, we strive to develop drugs that specifically neutralize these cells, which may help in addressing this unmet need in cancer therapy.

Motamedi Laboratory

Alex Gulka, BSc
Junichi Hanai, PhD*
Richard Joh, PhD
Jasbeer Khanduja, PhD
Jose Lombana, BSc
Mo Motamedi, PhD
Sunny Sharma, PhD

* Instructor in Medicine, HMS

Epigenetic changes are heritable, phenotypic alterations which occur without mutations to the underlying genes. Once triggered, these phenotypic changes persist through numerous cell divisions independently of the original inducing signal. Epigenetic changes are critical for the stable formation of cellular identities, upon which all developmental processes depend. Disruption to epigenetic regulation underlies a variety of human maladies, including cancers. In fact, epigenetic pathways can contribute to all stages of cancer progression, including initiation, metastasis, resistance and recurrence. Therefore, understanding the molecular mechanisms that establish epigenetic states is fundamental to the development of therapies that target the epigenetic components of cancers.

Often, but not always, epigenetic changes are concomitant with alterations to the chromatin state of underlying genes. Most of what is known about how chromatin states are altered in response to epigenetic triggers comes from decades of research in model organisms. These studies have revealed highly conserved protein families, which are now used for therapeutic or diagnostic purposes in cancers. The Motamedi lab uses the fission yeast as a model to understand how changes to eukaryotic chromatin are made, maintained and propagated, and how these changes establish alternative transcriptional programs particularly in response to persistent stress.

Noncoding RNAs and chromatin – partners in epigenetic regulation

One of the first models for how long and small noncoding RNAs regulate chromatin states was proposed in the fission yeast. It posits that noncoding RNAs, tethered to chromatin, provide a platform for the assembly of RNA-processing and chromatin-modifying proteins (Motamedi et al 2004), leading to transcriptional regulation of the underlying genes. In addition to acting as platforms, RNA molecules also can function as *trans*-acting factors, targeting chromatin regulatory proteins to specific chromosomal regions. These principles now have emerged as conserved mechanisms by which noncoding RNAs partake in chromatin regulation in eukaryotes including in humans.

The image depicts as cells enter quiescence (moon), they load Ago1 (ships) with euchromatic small RNAs to mediate Quiescent-induced Transcriptional Repression (Q) of a set of euchromatic genes. Exosome activity separates heterochromatic (dark blue) from euchromatic (yellow) regions. When entering quiescence, the exosome barrier opens, permitting euchromatic transcripts (differently colored dots) to become substrates for RNAi degradation. Ago1, acquiring new color (sRNAs) as it crosses the exosome barrier, targets Q to the corresponding color in euchromatin.

A focus of the lab is cellular quiescence (or G0). G0 is a ubiquitous cellular state in which cells exit proliferation and enter a state of reversible dormancy. Developmental programs, such as wound healing, or exposure to a variety of stress, such as starvation, can trigger entry into or exit from G0. G0 cells have distinct transcriptional programs through which they acquire new properties compared to their proliferative selves, including long life, thrifty metabolism and resistance to stress. Loss of G0 regulation results in defects in developmental and adaptive programs. How cells enter, survive and exit G0 is a critical question in basic biology, which is largely unexplored. To address this knowledge gap, we modeled G0 in fission yeast and showed that when cells transition to G0, new ncRNAs emerge which coopt and deploy constitutive heterochromatin proteins (histone H3 lysine 9 methyltransferase, Clr4/SUV39H) to several euchromatic gene clusters to regulate the expression of a set of developmental, metabolic and cell cycle genes. We show that this pathway is critical for survival and the establishment of the global G0 transcriptional program. This work revealed a new function of heterochromatin proteins and noncoding RNAs, which orchestrate the genome-wide deployment of heterochromatin factors in response to long-term stress. It also led to the proposal of several hypotheses that we are currently testing. Moreover, in collaboration with several groups, we have begun to test whether this pathway also plays an important role in cancer dormancy and treatment resistance.

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*Co-authors

†This paper was the cover story in *Molecular Cell* and featured in *Boston Magazine* (*http://www.bostonmagazine.com/sponsor-content/mgh-study-potentially-finds-the-achilles-heel-for-dormant-cancer-cells/*)

††This article was previewed in *Dev Cell*. 16: 630-632, 2009

†††This article was the cover story in *Cell*

Christopher Ott, PhD

Mutations in cancer cells lead to malfunctioning control of gene expression. **The Ott laboratory** is dedicated to discovering the gene expression control factors that are essential for leukemia and lymphoma cell survival. Discovery of these factors prompts further efforts in our group to design chemical strategies for the synthesis and deployment of prototype drugs targeting the aberrant mechanisms of gene control. Biologically, gene control factors represent compelling therapeutic targets for these cancers, as they are master regulators of cell identity. Yet despite this clear rationale, most are perceived as intractable drug targets owing to their large size, disordered shapes, and involvement in complex cellular circuits. Recent advances in gene editing technologies and discovery chemistry have advanced our capability to rapidly identify targetable aspects of gene control and methods to disrupt their function. We use these genetic and chemical tools to probe cancer cell circuitry and inform therapeutic hypotheses.

Ott Laboratory

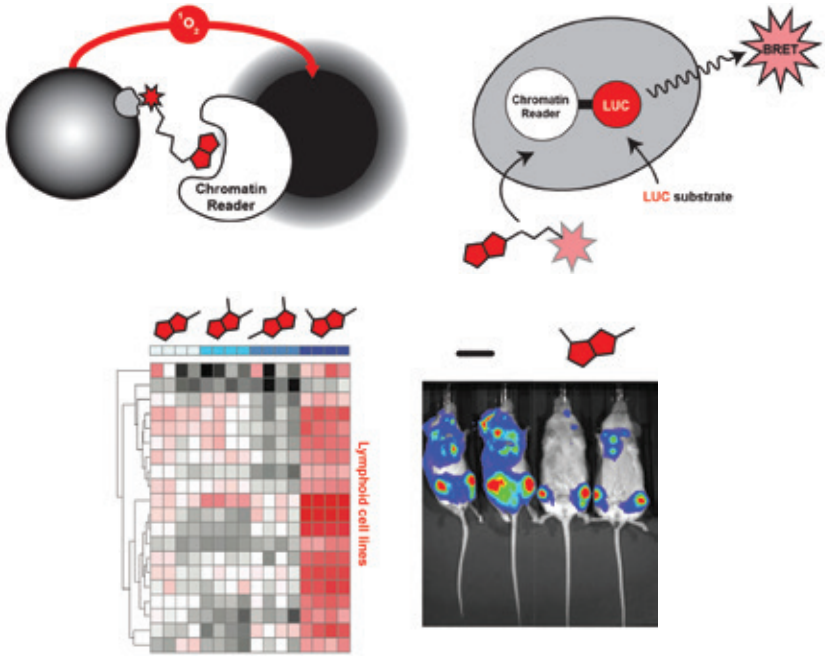
Manish Hudlikar, PhD
Matthew Lawlor
Christopher Ott, PhD
Sharon Wu

Chemical BET inhibition

Gene control factors bind to regions of transcriptionally active chromatin called enhancers. Enhancers are critical for driving cell-type specific gene expression, and their chromatin structures are typically marked with specific histone modifications. Among the most distinctive is lysine side-chain acetylation, recognized (or 'read') by histone modules called bromodomains. Recently, novel chemical compounds have been advanced that selectively target the bromodomains of the bromodomain and extra terminal domain (BET) family. These compounds efficiently displace BET proteins from active enhancer chromatin, and we and others have found them to be active agents in models of acute leukemia, lymphoma, and several solid tumor types. Using a suite of genome-wide chromatin and transcriptomic assays, we aim to understand principles of BET protein dependency in leukemia and lymphoma cells. Efforts are ongoing to establish biomarkers for response and resistance, and realize promising rationales for combination therapies with other targeted agents.

Essential enhancers

Classic studies have described oncogenic enhancers in leukemia and lymphoma cells. This aberrant enhancer activity can occur by chromosomal translocation of proto-oncogenes such as *MYC* and *BCL2* to the transcriptionally active immunoglobulin locus, T-cell receptor rearrangements in T cell tumors, and the *EVI1-GATA2* rearrangement in an aggressive subtype of acute myeloid leukemia. In addition to chromosomal translocations, cancer-specific enhancers have been described at proto-oncogene loci like *TAL1* and *MYC*, which are aberrantly bound by transcription factors through direct somatic mutation of enhancer DNA elements



Expanding the chromatin chemical probe toolbox with high throughput bead-based proximity assays, cellular target engagement assessment, cell line viability profiling, and in vivo pharmacology.

or focal amplification. We have generated high-resolution enhancer landscapes derived from primary patient samples, including a large cohort of chronic lymphocytic leukemia samples. Current projects include construction of core regulatory transcription factor circuitries, and the discovery of inherited and somatic variants leading to aberrant gene expression. Using genetic and epigenetic genome editing techniques, we are functionally dissecting malfunctioning enhancers and their cognate bound factors to derive mechanistic understanding of the essential enhancers principally responsible for maintaining leukemia and lymphoma cell states.

Expanding the chromatin chemical probe toolbox

The successful discovery of chemistry efforts that yielded efficient BET bromodomain inhibitors has revealed chromatin reader domains broadly, and bromodomains specifically, as protein modules amenable for small molecule ligand development.

Used experimentally, enhancer-targeting compounds enable precise disruption of chromatin features and can be used to identify and validate discrete biophysical and biochemical functions of target proteins. Paired with an understanding of integrated epigenomics, these probes enable the elucidation of fundamental insights into genome structure and function. We use high-throughput protein-protein interaction assays and cellular assays of chromatin reader activity to identify reader domain inhibitors. Lead compounds are iteratively optimized for potency and selectivity, followed by functional assessments on epigenome structure. Leukemia and lymphoma cell viability profiling and in vivo pharmacokinetic and pharmacodynamic studies enable the nomination of next-generation inhibitors of essential chromatin readers. Ongoing projects seek to expand our current toolbox of bromodomain inhibitors, with a particular focus on 'orphan' factors for which selective compounds have yet to be developed.

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*Co-corresponding authors

Shiv Pillai, MD, PhD

The Pillai laboratory asks questions about the biology of the immune system and susceptibility to disease. Some of these questions are 1) can we manipulate the immune system to treat autoimmunity and cancer and to increase immunological memory? 2) can we understand how genetics and the environment affect lymphoid clones to drive common diseases? and 3) can this latter information be used to better understand and develop new therapies for chronic inflammatory human diseases such as systemic sclerosis and IgG4-related disease? Our discovery of the role of an enzyme called Btk in the activation of B cells has contributed to the generation of Btk inhibitors that are effective in B cell malignancies and in trials of autoimmunity. One of the pathways we are currently studying suggests new approaches for the treatment of autoimmune disorders. We are also exploring novel ways to strengthen immune responses and enhance helper T cell memory that provide hope for developing more effective personalized immune-system based treatments for cancer.

Pillai Laboratory

Faisal Alsufyani, MD
Hugues Allard-Chamard, MD, PhD
Alice Bertocchi
Edwin Delfin
Jocelyn Farmer, MD, PhD
Kelsey Finn*
Naoki Kaneko, DDS, PhD
Hang Liu, MD
Marshall Karpel*
Jesper Kers MD, PhD
Vinay Mahajan, MD, PhD
Hamid Mattoo, PhD
Sam Murphy
Cory Perugino, DO
Shiv Pillai, MD, PhD
Na Sun
Grace Yuen, PhD

* PhD Candidate

Pathogenesis of fibrosis (NIAID Autoimmune Center of Excellence at MGH)

In studies on the immunology of IgG4 related disease and scleroderma, performed in collaboration with John Stone (MGH Rheumatology) and Dinesh Khanna, (U. of Michigan, Rheumatology), we have identified an unusual, clonally expanded and potentially "fibrogenic" human CD4+ effector T cell subset in affected tissues. The differentiation and protective role of these CD4+ CTLs in cancer and chronic viral infections are currently being investigated using chromatin accessibility mapping, DNA methylation studies and single cell RNA-seq approaches.

Studies on murine and human B cell development and activation

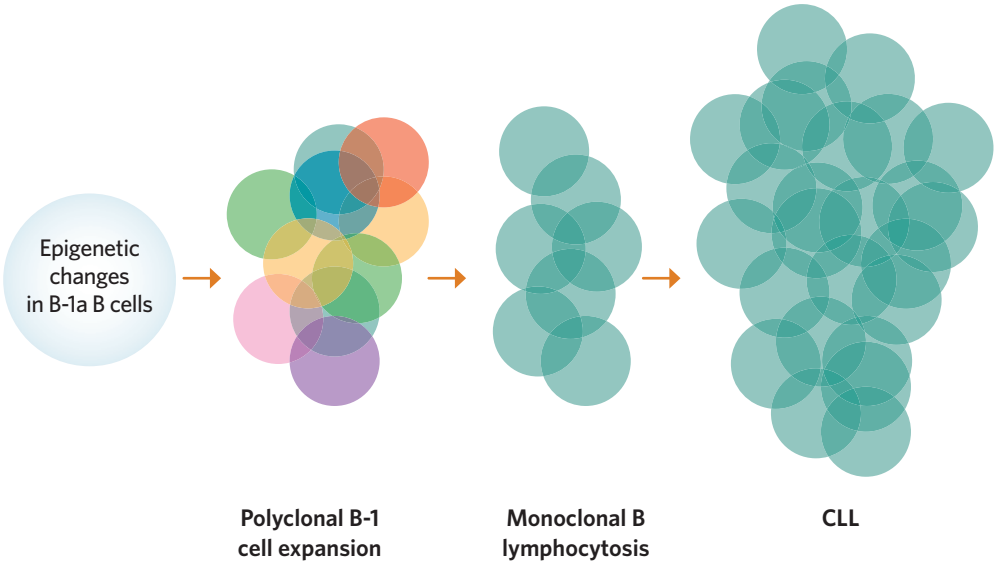
We are using a number of single cell transcriptomic, epigenetic and genetic approaches to examine the heterogeneity and development of murine and human B cells, as well as the molecular bases of the processes of T-B collaboration and germinal center formation.

DNA methylation, B cell self-renewal and chronic lymphocytic leukemia

We have long been interested in cell fate decisions in B cell development and in the development of self-renewing B cell subsets. The roles of DNMT3a in B-1a B cell self-renewal and of specific methylation events in chronic lymphocytic leukemia are being investigated.

Dock2 regulates T cell memory and T-B collaboration

We have identified Dock2 as a regulator of the strength of the immune response and the generation of CD8+ and CD4+ T cell memory. This gene also contributes the strength of the



A model for the evolution of CLL.

germinal center response. The inactivation of this gene leads to the clearance of intracellular pathogens and may enhance anti-tumor immunity.

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Luca Pinello, PhD

The focus of **the Pinello laboratory** is to use innovative computational approaches and cutting-edge experimental assays, such as genome editing and single cell sequencing, to systematically analyze sources of genetic and epigenetic variation and gene expression variability that underlie human traits and diseases. The lab uses machine learning, data mining and high performance computing technologies, for instance parallel computing and cloud-oriented architectures, to solve computationally challenging and Big Data problems associated with next generation sequencing data analysis. Our mission is to use computational strategies to further our understanding of disease etiology and to provide a foundation for the development of new drugs and novel targeted treatments.

Pinello Laboratory

Huidong Chen*
(shared with Guo-Cheng Yuan Lab)
Kendell Clement, PhD
Jonathan Hsu*
(shared with Keith Joung Lab)
Antonin Klima
Luca Pinello, PhD
Qiuming Yao, PhD

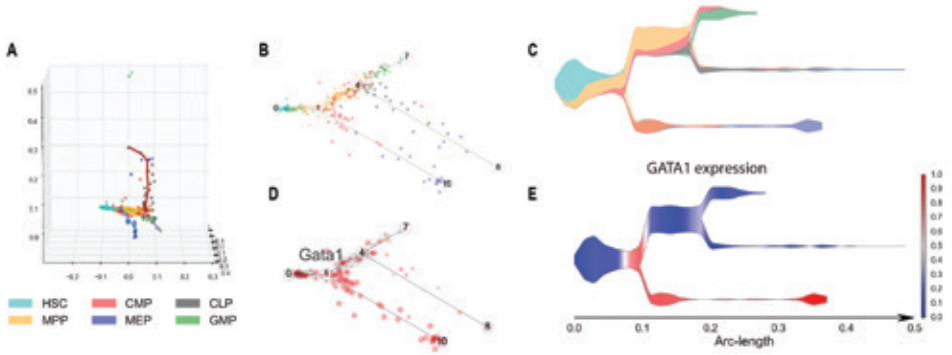
* PhD candidate

Epigenetic variability in cellular identity and gene regulation

We are studying the relationship between epigenetic regulators, chromatin structure and DNA sequence and how these factors influence gene expression patterns.
We recently proposed an integrative computational pipeline called HAYSTACK (*https://github.com/lucapinello/Haystack*). HAYSTACK is a software tool to study epigenetic variability, cross-cell-type plasticity of chromatin states and transcription factor motifs and provides mechanistic insights into chromatin structure, cellular identity and gene regulation. By integrating sequence information, histone modification and gene expression data measured across multiple cell-lines, it is possible to identify the most epigenetically variable regions of the genome, to find cell-type specific regulators, and to predict cell-type specific chromatin patterns that are important in normal development and differentiation or potentially involved in diseases such as cancer.

Computational methods for genome editing

Recent genome editing technologies such as CRISPR/Cas9 are revolutionizing functional genomics. However computational methods to analyze and extract biological insights from data generated with these powerful assays are still in an early stage and without standards. We have embraced this revolution by developing cutting-edge computational tools to quantify and visualize the outcome of CRISPR/Cas9 experiments. We created a novel computational tool called CRISPResso (*http://github.com/lucapinello/CRISPResso*), an integrated software pipeline for the analysis and visualization of CRISPR-Cas9 outcomes from deep sequencing experiments, as well as a user-friendly web application that can be used by non-bioinformaticians (*http://crispresso.rocks*). In collaboration with Daniel Bauer's and Stuart Orkin's groups, we recently applied CRISPResso and other computational strategies to aid the development of an *in situ* saturation mutagenesis approach for



ARIADNE on transcriptomic data from the mouse hematopoietic system. A) Dimensionality reduction, reconstructed hierarchical structure composed of curves approximating the inferred trajectories. Single cells are represented as circles and colored according to the FACS sorting labels. B) Flat tree representation at single cell resolution; branches are represented as straight lines, (cells are represented as in A). The length of the branches and the distances between cells and assigned branches are proportional to the original representation in the 3D space. C) Rainbow plot: intuitive visualization to show cell type distribution and density along different branches. D) Single cell resolution expression pattern of GATA1, each circle is red filled proportionally to the relative expression of GATA1 in the whole population. E) Relative expression of GATA1 in each branch using the representation in C.

dissecting enhancer functionality in the blood system with the aim of developing potential therapeutic genome editing applications for hemoglobin disorders.

Exploring single cell gene expression variation in development and cancer

Cancer often starts from mutations occurring in a single cell that results in a heterogeneous cell population. Although traditional gene expression assays have provided important insights into the transcriptional programs of cancer cells, they often measure a combined signal from a mixed population of cells and hence do not provide adequate information regarding subpopulations of malignant cells. Emerging single cell assays now offer exciting opportunities to isolate and study individual cells in heterogeneous cancer tissues, allowing us to investigate how genes transform one subpopulation into another. Characterizing stochastic variation at the single cell level is crucial to understand how healthy cells use variation to modulate their gene expression programs, and how these patterns of variation are disrupted in cancer cells. We are currently developing a method called ARIADNE to model the variability of gene expression at single cell resolution, and to reconstruct developmental trajectories (see illustrative image) using data from single cell assays such as scRNA-seq, multiplexed qPCR or sc-ATAC-seq. This method can be used for disentangling complex cellular types and states in development, cancer, differentiation or in perturbation studies.

Selected Publications:

Beyaz S*, Kim JH*, **Pinello L***, Xifaras ME, Hu Y, Huang J, Kerenyi MA, Das PP, Barnitz RA, Herault A, Dogum R, Haining WN, Yilmaz ÖH, Passegue E, Yuan GC, Orkin SH, Winau F. The histone demethylase UTX regulates the lineage-specific epigenetic program of invariant natural killer T cells. *Nat Immunol*. 2017 Feb;18(2):184-195.

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*Co-first authors

Esther Rheinbay, PhD

Most known genomic drivers of cancer are in coding genes, affecting the encoded protein's interaction with other proteins, DNA or biological compounds. Recent advances in DNA sequencing technology have made it possible to study non-coding regions that regulate these protein-coding genes. Several cancer drivers have been identified and characterized in these regulatory regions, however, this genomic territory remains relatively unexplored in human tumors. **The Rheinbay laboratory** concentrates on identifying and functionally characterizing these non-coding drivers in the sequences of tumor whole genomes through development of novel analysis strategies and collaborations with experimental investigators.
We are also interested in tumors, especially breast cancers, for which no known protein-coding driver alterations have been found. In the age of targeted therapy, these tumors pose a special challenge in that they leave few treatment options for patients beyond conventional chemotherapy. We believe that finding novel genomic and epigenomic, protein-coding and regulatory therapeutic targets in these tumors will have significant clinical implications.

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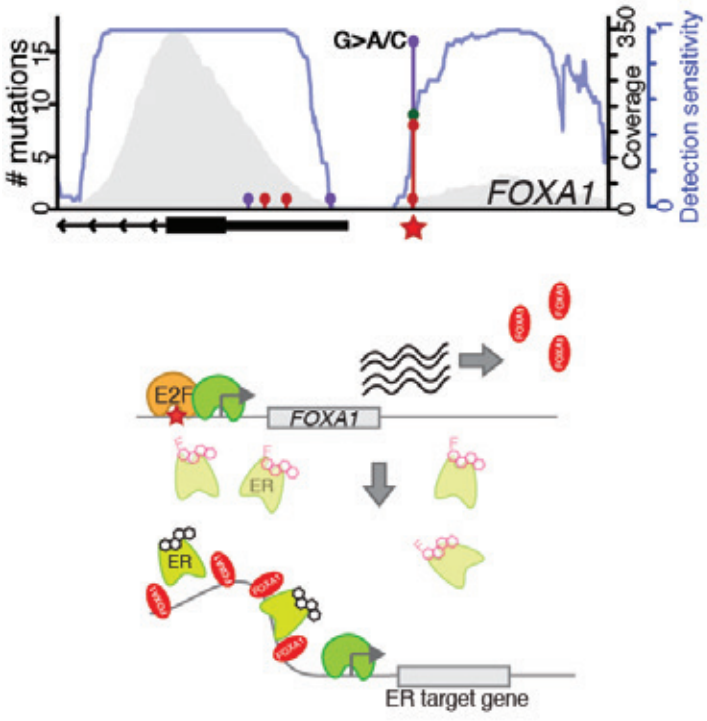
Rheinbay Laboratory

(opens Fall of 2018)
Esther Rheinbay, PhD

Regulatory driver mutations in cancer genomes

Genomic cancer driver discovery has traditionally focused on protein-coding genes (the human exome), and large-scale sequencing of these genes in thousands of tumors has led to the discovery of novel frequently altered genes. However, exome sequencing focused only on coding genes does not allow analysis of non-coding regions in the human genome. Protein-coding genes are regulated by several types of genomic elements that control their expression (promoters, distal enhancers and boundary elements), translation (5'UTRs) and mRNA stability (3'UTRs). Alterations in the DNA sequence of these elements thus directly affect the expression and regulation of the target gene. Several such non-coding elements have been identified as recurrently altered in human cancer, and functionally characterized, although these non-coding drivers appear infrequent compared to protein-coding oncogenes and tumor suppressors. One reason might be that gene regulation is highly tissue-specific, and therefore driver alterations in non-coding regions might create a fitness advantage in only a single tumor type. Finding such a specific driver requires a sufficient number of whole genomes from this tumor type. With recent advances in DNA sequencing technology and an increasing number of whole cancer genomes available for analysis, we are just starting to map out and characterize regulatory driver alterations. The Rheinbay laboratory works on the development of novel methods to identify non-coding driver candidates using genomic and epigenomic sources of information, and to understand their impact on tumor initiation, progression and treatment resistance through collaborations with experimental colleagues.



Hotspot mutation in the FOXA1 promoter in breast cancer and proposed mechanism of action.

We have recently identified a recurrent mutation in the promoter of the breast cancer oncogene FOXA1. This mutation increases expression through augmenting a binding site for E2F, leading to E2F protein recruitment. In addition, *FOXA1* overexpression leads to resistance to the breast cancer drug, fulvestrant. We are now investigating the implications and mechanism of action of this mutation in breast cancer progression and treatment resistance.

Finding targetable vulnerabilities in cancers without known drivers

From recent large genome and exome sequencing studies of different cancer types, it has become apparent that there are almost always patients whose tumors harbor no common driver alteration such as *BRAF* mutation in melanoma, *HER2* amplification, or hormone receptor expression in breast and prostate cancer. In an era of treatments targeting such alterations specific to a patient's cancer cells, a lack of potentially druggable cancer drivers severely limits the repertoire of available therapy options. Rather than being truly without any drivers, these tumors are likely driven by yet uncharacterized protein-coding or regulatory genomic alterations, or an oncogenic state induced and maintained by epigenetic changes. Our research is focused on finding the drivers and vulnerabilities of these particular tumors by integrating genomics and epigenomics data, with the ultimate goal of connecting patients to effective targeted treatments.

Selected Publications:

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Rheinbay E*, Suva ML*, Gillespie SM, et al. An aberrant transcription factor network essential for Wnt signaling and stem cell maintenance in glioblastoma. *Cell Reports*. 2013;3:1567-79.

*Equal contribution

Miguel N. Rivera, MD

Research in **the Rivera laboratory** focuses on using genomic tools to identify and characterize critical pathways in pediatric tumors and sarcomas. An important feature shared by these tumors is their strong association with developmental processes and with gene regulation mechanisms that control cell proliferation and differentiation. Our work combines the use of genomic technologies for the direct identification of gene regulation abnormalities in tumors with functional analysis of critical pathways in several model systems. Given that the mechanisms that drive pediatric tumors and sarcomas are poorly understood at present, we anticipate that our work will point to new therapies for these diseases.

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Rivera Laboratory

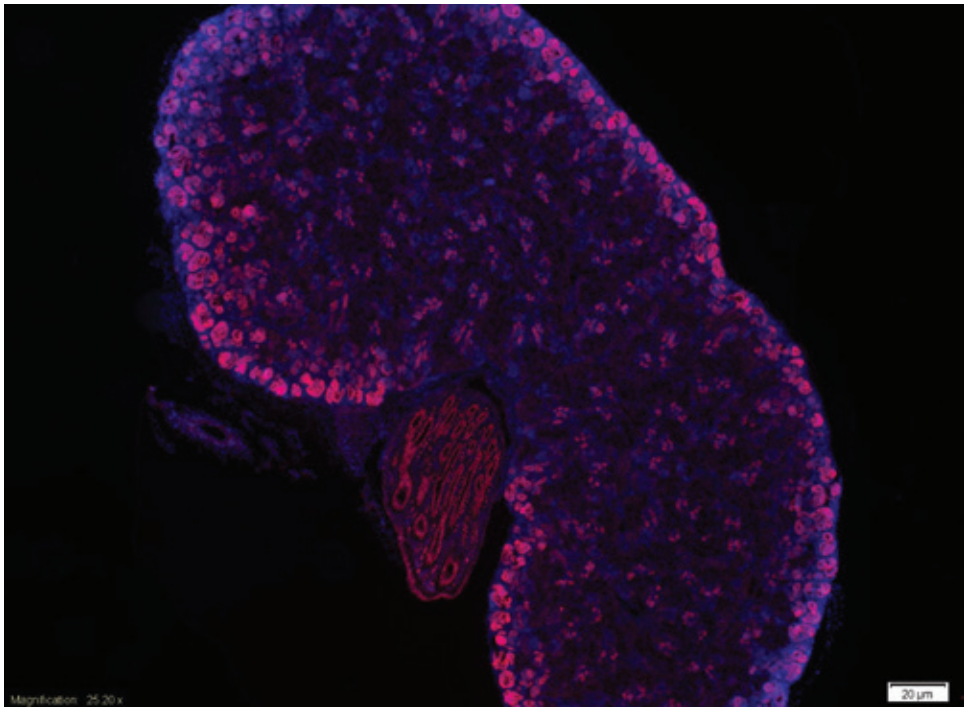
Alexandra Cauderay
Lukuo Lee
Miguel N. Rivera, MD
Angela Volorio
Yu-Hang Xing, PhD

Role of the WTX gene family in cancer and development

Wilms tumor, the most common pediatric kidney cancer, is a prime example of the connection between cancer and development, because it arises from kidney-specific stem cells and is composed of several cell types that resemble the earliest stages of kidney formation. We identified WTX, an X-linked tumor suppressor gene, which is inactivated in up to 30% of cases of Wilms tumor, by comparing the DNA of primary tumor samples with that of normal tissues using array comparative genomic hybridization (CGH). More recently, large tumor sequencing studies have shown that WTX is also inactivated in several other tumor types. WTX is the founding member of a new protein family (FAM123) and is expressed in the stem cells of the developing kidney, as well as in a variety of other tissues during embryogenesis. In collaboration with the Haber and Bardeesy laboratories, we have demonstrated that inactivation of WTX in mice leads to profound alterations in the development of several organs including kidneys, bones and fat by causing changes in the differentiation programs of mesenchymal stem cells. In particular, we observed an expansion of mesenchymal kidney stem cells, suggesting that WTX regulates the balance between proliferation and differentiation in these cells. We are now using a combination of in vitro and in vivo approaches to elucidate the molecular mechanisms by which WTX and related proteins regulate stem cells. Given that the same mechanisms are likely to be operative in tumors where WTX is inactivated, we expect that our studies may reveal new therapeutic opportunities for a variety of tumor types.

Epigenomic approaches for the identification of novel pathways in cancer

While genetic studies have led to the development of important cancer therapies, most genetic alterations in cancer do not point to specific therapeutic targets. In the case of pediatric cancers and sarcomas, which are often driven by low numbers of recurrent mutations, the identification of therapeutic targets through genetic studies has been particularly challenging. In order to discover new pathways involved in these tumors, we are using new genomic technologies to



Immunofluorescence image of a developing mouse kidney. The transcription factor Pax2 (red) is present in the stem cells that can give rise to Wilms tumor (adjacent to the surface of the organ) and in precursors to collecting ducts.

identify abnormalities in the mechanisms that regulate gene expression programs controlling cell proliferation and differentiation.

One of these technologies is genome-wide chromatin profiling, which combines chromatin immunoprecipitation and high-throughput sequencing. This approach has been used to study how genes are activated or repressed by regulatory elements in the genome such as promoters and enhancers. As a complement to gene expression studies, chromatin profiling provides a unique view of gene regulation programs by allowing the identification of both active and repressed genomic domains based on patterns of histone modification. Several studies have shown that prominent active histone marks are associated with genes that play key roles in cell identity and proliferation, including oncogenes that promote the growth of tumor cells. In contrast, repressive marks are found at loci that are maintained in an inactive state to prevent cellular differentiation.

In recent studies we have applied chromatin profiling to Wilms tumor, Ewing sarcoma and medulloblastoma, three pediatric tumors that are thought to arise from stem cell precursors and that have been linked to abnormalities in transcriptional regulation. Our work has uncovered novel genes and pathways involved in these diseases by comparing chromatin patterns in primary tumor samples and normal tissue specific stem cells. In addition, we have identified gene regulation mechanisms that play critical roles in tumor formation through functional studies of transcription factors and chromatin regulators. We are now characterizing these pathways in detail and extending our epigenomic analysis to other tumor types where oncogenic pathways are poorly defined.

Selected Publications:

Boulay G, Sandoval GJ, Riggi N, Iyer S, Buisson R, Naigles B, Awad ME, Rengarajan S, Volorio A, McBride MJ, Broye LC, Zou L, Stamenkovic I, Kadoch C, **Rivera MN**. Cancer-specific retargeting of BAF complexes by a prion-like domain. *Cell*. 171(1-16), 2017 Sept 21.

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*Co-authors

Dennis Sgroi, MD

The overarching goals of research in **the Sgroi laboratory** are to develop better ways to identify patients who are at risk for the development of breast cancer and to identify those breast cancer patients who are likely to benefit from targeted drug therapies. We are taking several different approaches to achieving these goals. First, we are deciphering specific molecular events that occur during the earliest stages of tumor development and using this knowledge to develop biomarkers that will predict for increased risk of progression to cancer. Second, using DNA microarray technologies, we are searching for novel breast cancer biomarkers to identify patients with hormone-receptor-positive breast cancer who are most likely to benefit from extended hormonal therapy. Finally, we are taking a combined approach—based on analysis of tissue from breast cancer patients and various laboratory studies—to identifying biomarkers that will predict how individual breast cancer patients will respond to novel targeted therapeutics.

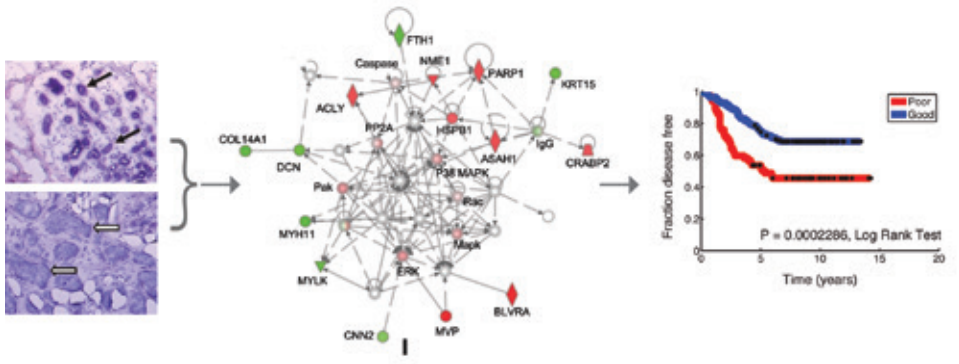
Sgroi Laboratory

Wayland Chiu
Dennis Sgroi, MD
Marinko Sremac, PhD

Our research focuses on understanding the molecular genetic events associated with the pathogenesis of human breast cancer. My laboratory has developed technological approaches to study gene expression in the earliest microscopic precursor lesions as well as in the latest stages of human breast cancer. Specifically, we have been successful in combining laser capture microdissection, high-density cDNA array, and real-time quantitative PCR (RTQ-PCR) technologies to identify novel gene expression patterns in human breast cancer. Using this approach, we have demonstrated for the first time that atypical intraductal hyperplasia and ductal carcinoma in situ are direct precursors to invasive ductal carcinoma. More specifically, we have shown that the various pathological stages of breast cancer progression are highly similar at the transcriptional level, and that atypical intraductal hyperplasia—the earliest identifiable stage of breast cancer—is a genetically advanced lesion with an expression profile that resembles that of invasive breast cancer. More recently, we have studied the gene expression changes of the stromal microenvironment during breast cancer progression, and we have demonstrated that the transition from preinvasive to invasive breast cancer is associated with distinct stromal gene expression changes.

Presently, my laboratory is focused on applying high-throughput molecular technologies to identify biomarkers that will predict the clinical behavior of human breast cancer in the setting of specific hormonal and chemotherapeutic regimens.

We have independently developed two complementary biomarkers—the Molecular Grade Index (MGI) and the HOXB13/IL17BR (H/I). MGI is a molecular surrogate for histological grade and a highly precise biomarker for risk of breast cancer recurrence. The HOXB13:IL17BR index, on the other hand, is a biomarker of endocrine responsiveness in ER+ breast cancer, as it has been shown to predict for benefit from adjuvant tamoxifen



The comparative analysis of the transcriptome and proteome of normal breast epithelium and malignant breast epithelium (top panel) combined with a proteome network analysis has led to the discovery of a novel robust network-based biomarker (center) with clinical relevance (right).

and extended adjuvant aromatase inhibitor therapy. Most recently, we demonstrated that the combination MGI and H/I, called the Breast Cancer Index (BCI), outperforms the Oncotype Dx Recurrence Score for predicting risk of recurrence. As a result of our collective data, we anticipate assessing BCI in clinical trials of extended adjuvant hormonal therapy. Given that HOXB13 expression in clinical breast cancers is associated with endocrine therapy responsiveness, we are currently investigating the functional activity of HOXB13 and assessing its possible role as a surrogate marker for a nonclassical estrogen receptor signaling pathway.

Selected Publications:

Zhang Y, Schroeder BE, Jerevall PL, Ly A, Nolan H, Schnabel CA, **Sgroi DC**. A Novel Breast Cancer Index For Prediction of Distant Recurrence in HR+ Early-Stage Breast Cancer with One to Three Positive Nodes. *Clin Cancer Res.* 2017 Dec 1;23(23):7217-7224.

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Sgroi DC, Carney E, Zarrella E, Steffel L, Binns SN, Finkelstein DM, Szymonifka J, Bhan AK, Shepherd LE, Zhang Y, Schnabel CA, Erlander MG, Ingle JN, Porter P, Muss HB, Pritchard KI, Tu D, Rimm DL, Goss PE. Prediction of Late Disease Recurrence and Extended Adjuvant Letrozole Benefit by the HOXB13/IL17BR Biomarker. *J Natl Cancer Inst.* 2013; 105:1036-1042.

Zhang Y, Schnabel CA, Schroeder BE, Jerevall PL, Jankowitz RC, Fornander T, Stal O, Brufsky AM, Sgroi D, Erlander M. Breast Cancer Index Identifies Early Stage ER+ Breast Cancer Patients at Risk for Early and Late Distant Recurrence. *Clin Cancer Res.* 2013 Aug 1;19(15):4196-205.

Toshihiro Shioda, MD, PhD

Shioda Laboratory

Molecular Profiling Laboratory

Keiko Shioda, RN, BS

Toshihiro Shioda, MD, PhD

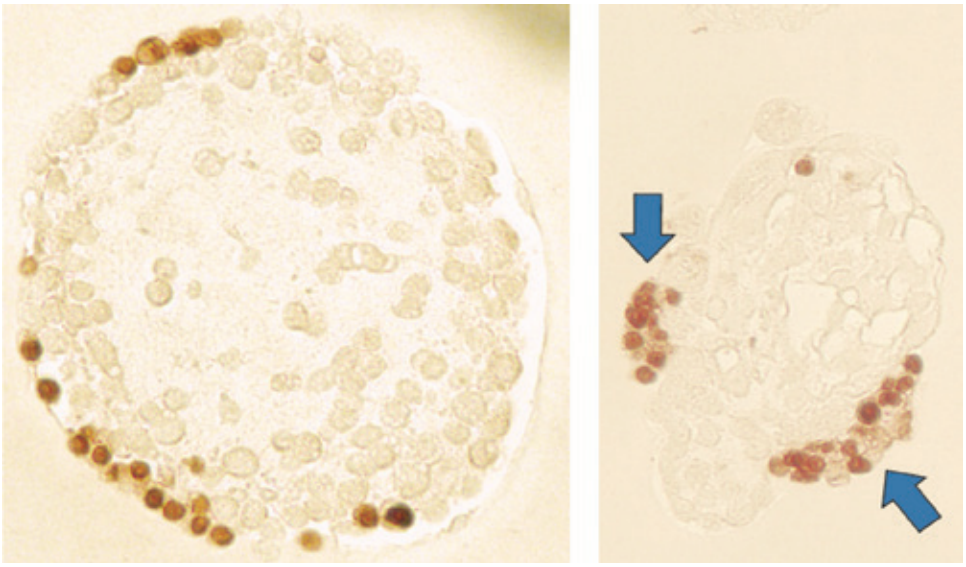
The Shioda laboratory is interested in biology and diseases of human germline, which is the specialized population of cells destined to generate sperm or eggs. The germline is solely responsible for conveying the entire genetic information to the next generation. Thus, all heritable, disease-causing genetic mutations occur only in the germline. The first germline cells, which are known as primordial germ cells (PGCs), are observed in human embryos during the third week of gestation as a cluster of only 40 cells, and this is the only single opportunity to generate the germline in each lifespan. Because of the extreme difficulty to obtain human PGCs for research, scientific knowledge of normal biology and mechanisms of genetic damages in human PGCs is very limited. To overcome this restriction, our laboratory has been generating PGC-like cell culture models from human induced pluripotent stem cells. Using these models, we attempt to examine how drugs or environmental factors can introduce disease-causing damages into the genome of human germline.

In Vitro Generation of Mammalian Primordial Germ Cells from Pluripotent Stem Cells

The germline is the specialized population of cells destined for gametogenesis and solely responsible for conveying genetic and epigenetic information to the subsequent generation. During the third week of gestation, human primordial germ cells (PGCs) are observed in the wall of yolk sac as a cluster of only 40 cells. While rapidly proliferating, PGCs migrate towards genital ridges, supported by the SCF/KIT survival signal and guided by the SDF1/CXCR4 chemotaxis signal. PGCs colonized in the genital ridges differentiate into sex-specific germline cells upon gonadal sex differentiation.

All heritable genetic aberrations, including cancer predisposition mutations, occur exclusively in the germline. There is also evidence that shows that *in utero* exposure of mammalian embryos and/or fetuses to various types of environmental stresses such as therapeutic drugs, toxic industrial chemicals, or malnutrition may cause trans-generationally heritable epigenetic changes that could convey predisposition to various adult-onset diseases, including obesity and malignancies. However, epigenetic marks are subjected to global and robust erasure in PGCs and then re-established during later stages of germline differentiation. How the transgenerational epimutations can escape this reprogramming process is unknown.

One of the major hurdles of studying mammalian germline is the difficulty to obtain sufficient amounts of early-stage germline cells from embryos or fetuses. Moreover, animal experiments using rodent models often result in total absence of germline or embryonic lethality after exposure to stresses or genetic manipulations. To overcome these problems, we produce and characterize PGC-like cell culture models (PGC-LCs for PGC-Like Cells) from human and mouse pluripotent stem cells to establish their usefulness in



Emergence of human PGC-LCs on the surface of embryoid bodies. Human PGC-LCs are visualized by anti-OCT4 immunohistochemistry of FFPE slides. Most PGC-LCs are localized in the outermost surface layer of embryoid bodies (left). In rare instances, PGC-LCs form clusters on the surface (arrows; right).

studying mechanisms, effects, and prevention of germline mutations or heritable epigenetic aberrations. Last year we published a study demonstrating very robust and global DNA demethylation in the genome of mouse PGC-LCs, resembling the epigenetic erasure process in embryonic PGCs. Several types of repetitive elements, such as the IAP class endogenous retroviruses, escaped the global epigenetic erasure in mouse PGC-LCs as well as embryonic PGCs. Mouse PGC-LCs were also able to erase iPSC-derived, aberrant DNA hypermethylation at the *Dlk1-Dio3* imprinting control region, and this is the first experimental demonstration that a specific epimutation is erased during the epigenetic reprogramming in the germline. To distinguish the epigenetic characteristics between paternally and maternally derived alleles, we have established mouse iPSC clones whose paternal and maternal chromosomes are derived from *Mus spretus* and *Mus musculus*, respectively. Taking advantage of their rich and evenly distributed SNPs, we are presently developing a computational data analysis pipeline for sensitive and quantitative determination of allele-biased gene expression and epigenetic marks in these iPS cells and their PGC-LC products.

Extending our PGC-LC studies, we have established a novel protocol for robust and highly reproducible production of human PGC-LCs from iPSCs. We have demonstrated a well-conserved transcriptomal signature of human PGC-LCs produced in multiple independent laboratories including ours, thus contributing to establish a foundation of practical applications of this novel cell culture model. Our ongoing study has been accumulating evidence that human PGC-LCs resemble embryonic PGCs in the early migrating stage, during which PGCs receive the SCF/KIT survival signal but not the SDF1/CXCR4 chemotactic signal yet. Because this stage of PGCs have been presumed as a precursor of the extra-gonadal germ cell tumors, we are trying to reconstitute the oncogenic process of germ cell tumors by introducing known cancer predisposition mutations into human PGC-LCs. Attempts are also being made to study mechanisms through which therapeutic drugs or toxic chemicals introduce genetic mutations and/or epigenetic aberrations in the genomes of human and mouse PGC-LCs.

Selected Publications:

Shoucri BM, Matinez ES, Abreo TJ, Hung VT, Moosova Z, **Shioda T**, and Blumberg B. Retinoid X receptor activation alters the chromatin landscape to commit mesenchymal stem cells to the adipose lineage. *Endocrinology*.

Miyoshi N, Stel JM, **Shioda K**, Qu N, Odajima J, Mitsunaga S, Zhang X, Nagano M, Hochedlinger K, Isselbacher KJ, and Shioda T. Erasure of DNA methylation, genomic imprints, and epimutations in a primordial germ-cell model derived from mouse pluripotent stem cells. *Proc Natl Acad Sci U S A*. 2016; 113(34):9545-5.

Miyoshi N, Wittner BS, **Shioda K**, Hirota T, Ito T, Ramaswamy S., Isselbacher KJ, Sgroi DC, and Shioda T. Nodes-and-connections RNAi knockdown screening: Identification of a signaling molecule network involved in fulvestrant action and breast cancer prognosis. *Oncogenesis*. 2015; 4:e172.

National Research Council Committee (Berg AO, Bailor III JC, Gandolfi AJ, Kriebel D, Morris JB, Pinkerton KE, Rusyn I, **Shioda T**, Smith TJ, Wetzler M, ZeiseL, and Zweidler-McKay P). Review of the Formaldehyde Assessment in the National Toxicology Program 12th Report on Carcinogens. The National Academies. *The National Academies Press*. 2014.

Janesick AS, **Shioda T**, Blumberg B. Transgenerational inheritance of prenatal obesogen exposure. *Molecular and Cellular Endocrinology*. 2014; 398:31-35.

David Spriggs, MD

The Spriggs laboratory has been focused on the proteins on the ovarian cancer cell surface and how those proteins regulate function in health and cancer. The serum cancer marker , CA125, has been used to manage ovarian cancer since the 1980's but its function is not known. CA125 has been identified as the product of the tethered mucin MUC16. Our studies over the past several years have provided insights into the function of MUC16. It is now apparent that the MUC16 regulates functions like growth, invasion and metastatic disease through the structure of sugars (glycosylation) on the surface of normal and cancer cells. This regulation requires interaction with specialized sugar binding proteins, Galectins which are key components of the tumor microenvironment. We are actively developing our antibodies against MUC16 and Galectin 3 for diagnosis, imaging and treatments. Our work has shown that antibodies which inhibit these cell – cell interactions can slow tumor growth and block the spread of cancer cells locally and inhibit the spread to new organs.

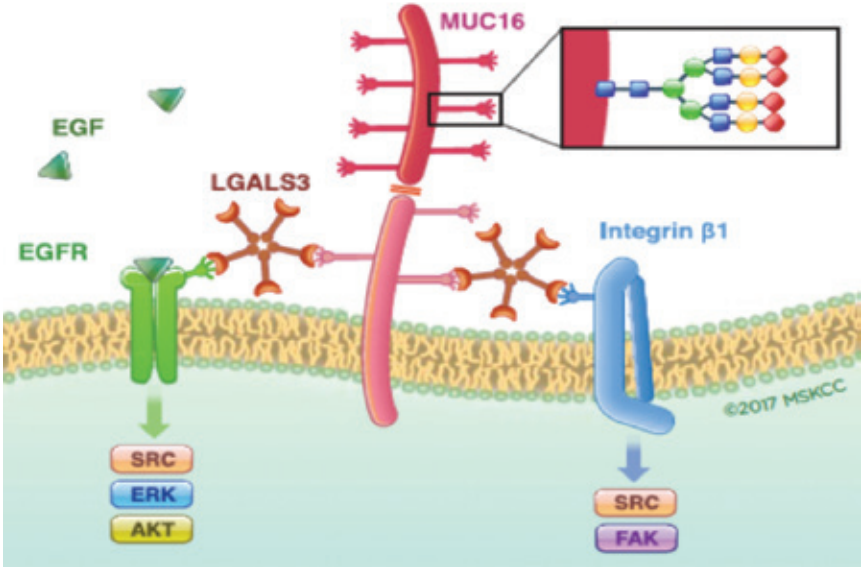
Spriggs Laboratory

Ian Caster, BS
Uyeh Ho, BS
David Spriggs, MD
Olapado Yeku, MD, PhD

Our research group is actively examining the role of glycosylation in tumor specific behaviors including uncontrolled growth, oncogene activation, invasion, immune system evasion angiogenesis, and metastatic spread. This work includes both inhibitory antibodies against MUC16 and Galectin-3 as sutiable clinical targets.

Anti-MUC16 biology Our current MUC16 work concentrates on development of our human MUC16 antibodies for targeting ovarian cancer. Our antibodies uniquely target the most proximal, retained portion of the MUC16 following cleavage and release of the CA125 antigen into the circulation. This retained ectodomain is a 58 amino acid peptide, linked to the membrane via a short transmembrane domain and a 31 amino acid cytoplasmic tail which is linked to the cellular cytoskeleton for mobility. The introduction of elements from MUC16 We have shown that most of the adverse consequences related to MUC16 expression. In fact, as little as 114 amino acids from the carboxyl terminal of the intact MUC16 sequence is sufficient to transform immortalized 3T3 mouse fibroblasts. This transformation results in increased soft agar colony formation, Matrigel invasion with increased MMP2/MMP9 expression, activation of both AKT and ERK proto-oncogenes and enhanced growth in nude mice. Similar effects are observed in MUC16 negative ovarian cancer cells. Deletion experiments demonstrate that the MUC16 ectodomain is required for this effect but loss of the cytoplasmic domain is dispensable. If one examines the ectomain in greater detail, it is found to be highly homologous with MUC16 analogs in most mammals particularly the portion of the sequence containing 2 N-glycosylation sites.

Glycosylation Dependence Our work has been the first to shown that the oncogenic effects of MUC16 require MGAT5 dependent tetra-antennary glycosylation of the MUC16 ectodomain and interaction with Galactin



Caption needed

3 (LGALS3). This complex then binds to glycosylation sites on growth factors including EGFr, Integrins and immune receptors like CTLA4. This has provided us with opportunities for MUC16+ cancer cell targeting.

Anti-MUC16 antibodies: Based on this information, we have constructed a panel of anti-MUC16 antibodies to target portions of the ectodomain. Antibodies against the ectodomain confirm that it is co-located in with common growth factor receptors in lipid rafts, and undergoes internalization / cycling. Antibodies that block the N-Glycosylation sites on the ectodomain effectively block the oncogenic properties of MUC16. We are now adapting our murine anti-MUC16 antibodies for 1) direct ovarian cancer targeting; 2) ADC with toxins like MMAE linked to humanized anti-MUC16 antibodies; 3) Bispecific T-cell engaging constructs linking MUC16 expressing cells to CD3 + T cells; 4) T cells directed at MUC16 epitopes.

MUC16-directed Chimeric Antigen Receptor (CAR) T Cells. Chimeric Antigen Receptor (CAR) T cells have not been successful in the management of solid tumor malignancies. Reasons for this include; poor trafficking, the presence of an immunosuppressive tumor microenvironment, CAR T-cell dysfunction and immune escape via antigen-loss. In conjunction with Olapado Yeku, a newly recruited faculty member and former MSKCC collaborator, we are using our antibodies as MUC16 targeted CAR T cells. We are developing strategies to further modify CAR T cells to optimize their efficacy for ovarian cancer and gynecologic malignancies. Our approaches to further engineering these CAR T cells are informed by the ovarian cancer tumor microenvironment. Using syngeneic immune competent mouse models and subsequent validation in genetically engineered and xenograft models, we are able to effectively evaluate these rationally optimized CAR T cells as monotherapy or in combination with other immunomodulatory agents prior to initiation of clinical trials.

Galectin 3 Targeting LGALS3 regulates the interaction of surface proteins with the extracellular membrane domain and mediates a signal cascade leading to invasion, oncogene activation and growth. While anti-MUC16 glycosylation site antibodies inhibit oncogenic properties, LGALS3 represents a more

Selected Publications:

{Rao, 2017 #8;Rao, 2011 #35;Rao, 2015 #45;Dharma Rao, 2010 #129;Chekmasova, 2010 #238;Park, 2008 #470;Yeku , 2017 #698}

Yeku OO, Purdon TJ, Koneru M, **Spriggs D**, Brentjens RJ. Armored CAR T cells enhance antitumor efficacy and overcome the tumor microenvironment. *Sci Rep*. 2017 Sep 5;7(1):10541.

Rao TD, Fernández-Tejada A, Axelrod A, Rosales N, Yan X, Thapi S, Wang A, Park KJ, Nemieboka B, Xiang J, Lewis JS, Olvera N, Levine DA, Danishefsky SJ, **Spriggs DR**. Antibodies Against Specific MUC16 Glycosylation Sites Inhibit Ovarian Cancer Growth. *ACS Chem Biol.* 2017 Aug 18;12(8):2085-2096.

Rao TD, Tian H, Ma X, Yan X, Thapi S, Schultz N, Rosales N, Monette S, Wang A, Hyman DM, Levine DA, Solit D, **Spriggs DR**. Expression of the Carboxy-Terminal Portion of MUC16/CA125 Induces Transformation and Tumor Invasion. *PLoS One*. 2015 May 12;10(5):e0126633.

Rao TD, Rosales N, **Spriggs DR**. Dual-fluorescence isogenic high-content screening for MUC16/CA125 selective agents. *Mol Cancer Ther.* 2011 Oct;10(10):1939-48.

Chekmasova AA, Rao TD, Nikhamin Y, Park KJ, Levine DA, **Spriggs DR**, Brentjens RJ. Successful eradication of established peritoneal ovarian tumors in SCID-Beige mice following adoptive transfer of T cells genetically targeted to the MUC16 antigen. *Clin Cancer Res.* 2010 Jul 15;16(14):3594-606.

Dharma Rao T, Park KJ, Smith-Jones P, Iasonos A, Linkov I, Soslow RA, **Spriggs DR**. Novel monoclonal antibodies against the proximal (carboxy-terminal) portions of MUC16. *Appl Immunohistochem Mol Morphol.* 2010 Oct;18(5):462-72.

Shannon Stott, PhD

The Stott laboratory is comprised of bioengineers and chemists focused on translating technological advances to relevant applications in clinical medicine. Specifically, we are interested in using microfluidics, biomaterials and imaging technologies to create tools that increase our understanding of cancer biology and of the metastatic process. The Stott laboratory has co-developed innovative microfluidic devices that can isolate extraordinarily rare circulating tumor cells (CTCs) from the blood of cancer patients. New microfluidic technologies are being developed for the isolation of other blood-based biomarkers such as exosomes and microvesicles as well as DNA. Ultimately, we hope that by working in close partnership with the molecular and cell biologists at the Mass General Cancer Center, we can create new tools that directly impact patient care.

Stott Laboratory*

Berent Aldikacti, MD
Uyen Ho
Michelle Jewett
Felis Koo, MS
Carmen Martin Alonso
Avanish Mishra, PhD*
Jacqueline Ohmura, PhD**
João Paulo Oliveira-Costa, PhD
Daniel Rabe, PhD
Derin Sevenler, PhD*
Shannon Stott, PhD
Shannon Tessier, PhD***
Rohan Thakur
Jessica Wallace
Mahnaz Zeinali

*Co-mentored with Mehmet Toner, PhD
**Co-mentored with Genevieve Boland, MD, PhD
***Instructor

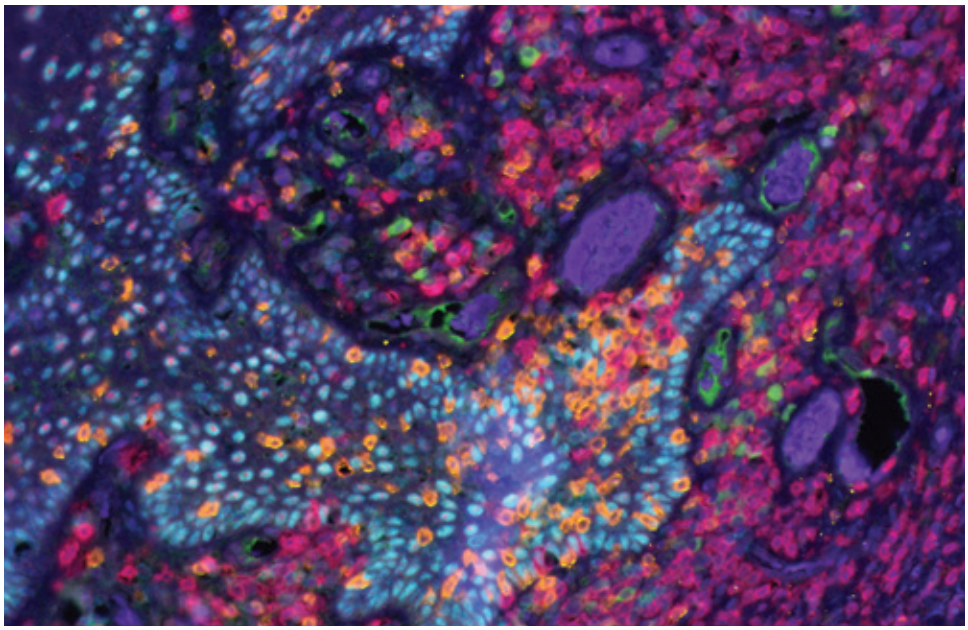
Rapid technological advances in microfluidics, imaging and digital gene-expression profiling are converging to present new capabilities for blood, tissue and single-cell analysis. Our laboratory is interested in taking these advances and creating new technologies to help build understanding of the metastatic process. Our research focus is on 1) the development and application of microfluidic devices and biomaterials for the isolation and characterization of extracellular vesicles, 2) the enrichment and analysis of CTCs using microfluidics, and 3) novel imaging strategies to characterize cancer cells, CTC clusters and extracellular vesicles.

Extracellular Vesicle Isolation and Characterization

Extracellular vesicles (EVs), such as exosomes, microvesicles, and oncosomes, are small particles that bud off of cancer cells, with some cancer cells releasing up to thousands of EVs per day. Researchers have hypothesized that these EVs shed from tumors transport RNA, DNA and proteins that promote tumor growth, and studies have shown that EVs are present in the blood of most cancer patients. Ongoing work in my lab incorporates microfluidics and novel biomaterials to enrich tumor-specific EVs from the plasma of glioblastoma and pancreatic cancer patients, using as little as 1mL of plasma. Once isolated, we are exploring their protein and nucleic acid content to probe their potential as a less invasive biomarker.

Microfluidics for Circulating Tumor Cell Analysis

One of the proposed mechanisms of cancer metastasis is the dissemination of tumor cells from the primary organ into the blood stream. A cellular link between the primary malignant tumor and the peripheral metastases has been established in the form of CTCs in peripheral blood. While extremely rare, these cells provide a potentially accessible source for early detection, characterization and monitoring of cancers that would otherwise require invasive serial biopsies. Working in collaboration with Drs. Mehmet Toner, Shyamala Maheswaran and Daniel Haber, we have designed a high throughput



Multispectral image of a tumor specimen from a head and neck patient.

Image courtesy of Joao Oliveira Da Costa, PhD

microfluidic device, the CTC-Chip, which allows the isolation and characterization of CTCs from the peripheral blood of cancer patients. Using blood from patients with metastatic and localized cancer, we have demonstrated the ability to isolate, enumerate and molecularly characterize putative CTCs with high sensitivity and specificity. Ongoing projects include translating the technology for early cancer detection, exploring the biophysics of the CTC clusters, and the design of biomaterials for the gentle release of the rare cells from the device surface. We are also developing new strategies for the long term preservation of whole blood such that samples can be shipped around the world for CTC analysis.

High-Content and High-Throughput Imaging of Cancer Cells

Cancer cells can be highly heterogeneous, with rare metastasis precursors capable of giving rise to a metastatic lesion mixed in with other tumor cells undergoing apoptosis. Thus, due to this heterogeneity, quantitative, robust analysis for individual cells may be critical for determining a particular cancer cells' clinical relevance in different disease contexts. Due to limitations in the number of distinct spectra that can be used in wide-field fluorescence imaging, high throughput characterization of cells and tissue is traditionally done with three to four colors. Our lab is exploring alternative imaging modalities, such as multi-spectral imaging (MSI), to enable quantitative analysis of multiple markers on a single cell. We are interested in using this technology to interrogate signaling activity in cancer cells. These data will be used to gain an increased understanding in the relationship between pharmacologic measurements and clinical outcomes, ultimately leading to the optimization of patient therapy.

Selected Publications:

Reátegui E*, van der Vos KE*, Lai CP*, Zeinali M, Atai NA, Floyd FP, Khankhel A, Thapar V, Toner M, Hochberg FH, Carter B, Balaj L, Ting DT, Breakefield XO, **Stott SL**, "Engineered Nanointerfaces for Microfluidic Isolation and Molecular Profiling of Tumor-specific Extracellular Vesicles", *Nat. Comm.* 2018; 9(1)

Wong KHK*, Tessier SN*, Miyamoto D, Miller KL, Bookstaver LD, Carey TR, Stannard C, Tai EC, Vo KD, Sandlin RD, Thapar V, Sequist LV, Ting DT, Haber DA, Maheswaran S, **Stott SL**†, Toner M†. Whole blood stabilization for precision oncology: isolation and molecular characterization of circulating tumor cells. *Nat. Comm.* 2017; 8(1)

Park MH*, Reátegui E*, Li W, Jensen AE, Toner M, **Stott SL**†, Hammond PT†, "Enhanced Isolation and Release of Circulating Tumor Cells Using Nanoparticle Binding in a Microfluidic Chip Via Place-Exchangeable Ligands", *JACS*. 2017; 139(7).

Au SH, Storey BD, Moore JC, Tang Q, Chen Y-L, Sarioglu AF, Javaid S, Langenau DM, Haber DA, Maheswaran S, **Stott SL**†, Toner M†, "Clusters of circulating tumor cells traverse capillary-sized vessels" *PNAS*. 2016; 113 (18).

Reátegui E. Aceto N. Lim EJ, Sullivan JP, Jensen AE, Zeinali M, Martel JM, Aranyosi AJ, Li W, Castleberry S, Bardia A, Sequist L.V, Haber D A, Maheswaran S, Hammond PT, Toner M, **Stott SL**. "Nanostructured coating for immunoaffinity capture and selective release of single viable circulating tumor cells" *Advanced Materials*. 2015; 27 (9).

Stott SL*, Hsu C-H*, Tsukrov DI, Yu M, Miyamoto DT, Waltman BA, Rothenberg SM, Shah AM, Smas ME, Korir GK, Floyd Jr. FP, Gilman A, Lord JB, Winokur D, Springer S, Irimia D, Nagrath SN, Sequist LV, Lee RJ, Isselbacher KJ, Maheswaran S, Haber DA, Toner M, Isolation of circulating tumor cells using a microvortex-generating herringbone-chip, Proc Natl Acad Sci. *PNAS*. 2010; 107 (43): 18392-19397.

*Co-authors, †Joint corresponding

Mario L. Suvà, MD, PhD

The Suvà laboratory is focused on developing and applying single-cell genomic technologies to dissect the biology of brain tumors, in particular adult and pediatric gliomas. We study patient samples at single-cell resolution and establish genetically and epigenetically relevant cellular models directly from clinical tumors. We model how brain cancer cells exploit their plasticity to establish phenotypically distinct populations of cells, with a focus on programs governing glioma stem cells. We seek to redefine tumor cell lineages and stem cell programs across all types of gliomas, and to leverage the information for renewed therapeutic attempts in gliomas. Through collaborations, the laboratory is invested in similar efforts in other types of tumors and participates in the tumor cell atlas and the human cell atlas initiatives.

Suvà Laboratory

Nick Gonzalez-Castro, MD, PhD
Simon Gritsch, MD, PhD
Henriette Mandelbaum*
Masashi Nomura, MD, PhD
Liz Perez*
Alyssa Richman, BS
Marni Shore, BS
Mario Suvà, MD, PhD
Hannah Weisman, BS'
Yixin Zhang, BS

*Graduate student

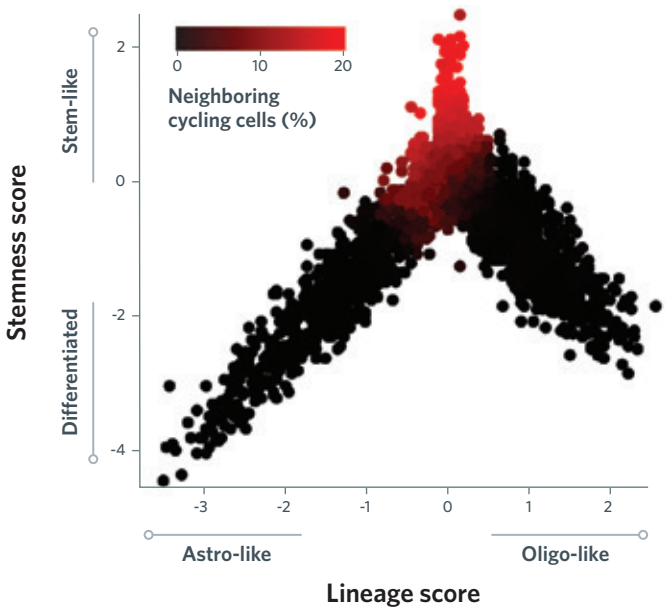
Gliomas are heterogeneous disease in which intra-tumoral heterogeneity contributes to disease progression and therapeutic failure. Glioma cells vary in stemness, proliferation, invasion, chemoresistance, apoptosis, and metabolism. Various factors contribute to this heterogeneity, on the one hand, branched genetic evolution of cancer cells generates distinct tumor sub-clones; on the other hand, it is also becoming increasingly clear that gliomas cells display functional properties related to developmental pathways and transcriptional programs, such as those associated with the self-renewal of tissue stem cells and their differentiation into specialized cell types. In order to dissect those influences and obtain a comprehensive view of gliomas biology, my laboratory is leveraging single-cell expression profiling across the spectrum of human gliomas, directly in patient samples. Analysis of transcriptomes of individual cells from human malignancies indeed offers a compelling approach to dissect the cellular state and infer partial genetic information from cancer cells in an unbiased way. We seek to discover novel therapies for gliomas.

Assessing Malignant Cells Heterogeneity at the Single-Cell Level in Gliomas.

Tumor heterogeneity poses a major challenge to cancer diagnosis and treatment. It can manifest as variability between tumors, or within cells from the same tumor, that may harbor different mutations or exhibit distinct phenotypic or epigenetic states. Such intra-tumoral heterogeneity is increasingly appreciated as a determinant of treatment failure and disease recurrence. The Suvà Lab is performing large-scale single-cell RNA-seq analyses in IDH-mutant gliomas, histone H3-mutant midline gliomas, IDH-wildtype glioblastoma, and medulloblastoma to assess tumor cell lineages, stem cell programs and genetic heterogeneity at an unprecedented scale and depth (see figure). Our goal is to identify both lineage-defined and somatically-altered therapeutic targets.

Dissecting the Ecosystem of Gliomas

The composition of the tumor micro-environment (TME) has an important impact on tumorigenesis and modulation of treatment responses. For example,



Single-cell analyses in oligodendrogliomas reveal a developmental hierarchy. Lineage scores (oligodendrocytic-like vs. astrocytic-like expression program; X-axis) and stemness scores (stem-like vs. differentiation expression program; Y-axis) of 3,348 single cancer cells from patient tumors. Each cell is color-coded based on the fraction of neighboring cells that is cycling. These findings suggest that undifferentiated cancer stem cells are the only compartment fueling growth of oligodendrogliomas in patients.

gliomas contain substantial populations of microglia and macrophages, with putative immunosuppressive functions but whose precise programs remains uncharted at single-cell resolution. In addition, very little is known about the functional state of T cells in human gliomas. As is the case in diverse other conditions, the CNS may create a unique microenvironment that impacts T cell function by distinct mechanisms. The laboratory leverages single-cell analyses in clinical samples to dissect the functional programs of immune cells in gliomas that can be used to elucidate mechanisms relevant to immuno-oncology. We profile both dysfunctional T cells that express multiple inhibitory receptors and T cells that are functional based on expression of multiple genes required for T cell cytotoxicity. We find these modules to be distinct from observations in other types of tumors (such as melanoma), underscoring the necessity to perform these analyses directly in gliomas. By analyzing modules of co-expressed genes in subsets of T cells in patients with glioma we seek to shed light on mechanism of activation and exhaustion in patient tumors and to highlight candidate novel regulatory programs that can be exploited for therapeutics.

Selected Publications:

Filbin MG†, Tirosh I†, Hovestadt V†, Shaw ML, Escalante LE, ..., Getz G, Rozenblatt-Rosen O, Wucherpfennig KW, Louis DN, Monje M, Slavc I, Ligon KL, Golub TR, Regev A*, Bernstein BE*, **Suvà ML*** Developmental and oncogenic programs in H3K27M gliomas dissected by single-cell RNA-seq. *Science*, 2018 Apr 20;360(6386).

Venteicher AS†, Tirosh I†, Hebert C, Yizhak K, Neftel C, Filbin MG, Hovestadt V, ..., Cahill DP, Rozenblatt- Rosen O, Louis DN, Bernstein BE, Regev A*, **Suvà ML***. Decoupling genetics, lineages and micro-environment in IDH-mutant gliomas by single-cell RNA-seq. *Science.* 2017 Mar 31; 55(6332).

Tirosh I†, Venteicher AS†, Hebert C, Escalante LE, Patel AP, Yizhak K, Fisher JM, ..., Rivera MN, Getz G, Rozenblatt-Rosen O, Cahill DP, Monje M, Bernstein BE, Louis DN, Regev A*, **Suvà ML***. Single-cell RNA-seq supports a developmental hierarchy in human oligodendroglioma. *Nature.* 2016 Nov 10;539(7628).

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*Co-senior authorship
†Co-first authorship

David A. Sweetser, MD, PhD

The Sweetser laboratory investigates how leukemia and other cancers develop with the goal of developing novel, safer, and more effective therapies. We are investigating how the Groucho/TLE family of co-repressors function as potent tumor suppressors of acute myeloid leukemia and their roles in normal development and cell function. Knock-out mice for Tle1 and Tle4 have identified critical roles for these proteins in hematopoiesis, bone, lung, and brain development, as well as a critical role in limiting inflammation. We have defined critical inflammatory signaling pathways mediating cell proliferation and synergistic cross talk within the cancer niche. The laboratory is also using whole exome sequencing to characterize underlying cancer predisposition genes in patients with a variety of pediatric malignancies. As the MGH site director for the newly established HMS Undiagnosed Diseases Center and Chief of Medical Genetics and Metabolism at MGH, Dr. Sweetser is also leading a group of clinicians and researchers actively engaged in elucidating the underlying basis of a wide variety of human diseases.

Sweetser Laboratory

Carl Holland, PhD
David A. Sweetser, MD, PhD

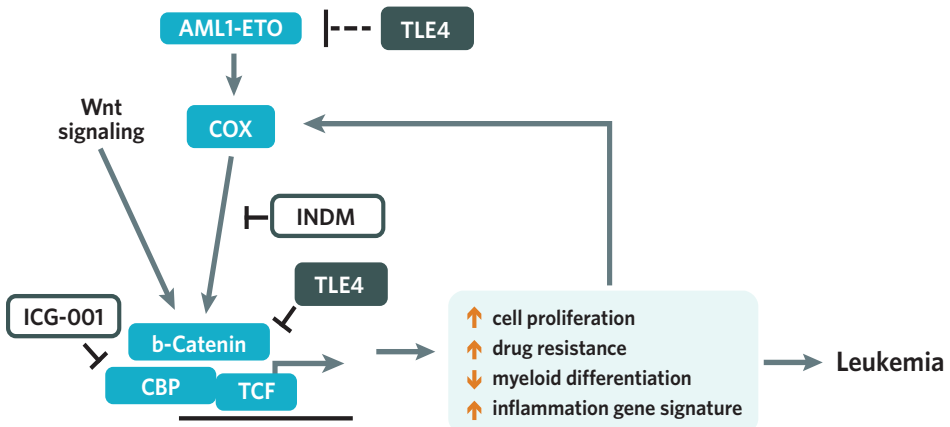
Genetics of Acute Myeloid Leukemia

Our laboratory is working to elucidate cooperating networks underlying leukemogenesis and to help develop novel targeted therapies for cancer. Current projects are detailed below.

Evaluation of the Role of the Groucho/TLE Family of Corepressors in Development and Cancer.

Our laboratory has defined TLE1 and TLE4 as members of a novel family of tumor suppressor genes, the TLE/Groucho proteins, the inactivation of which appears to be a key cooperating event with other oncogenes in the development of a subset of acute myeloid leukemias.

The Groucho/TLE family of corepressor proteins is known to modulate many of the major pathways involved in development and oncogenesis, including Wnt/β-catenin, Notch, Myc, NFκB, and TGFβ. However, researchers are only beginning to understand their potential role in oncogenesis. These genes appear to behave as tumor suppressor genes in the pathogenesis of other myeloid malignancies and lymphomas. However, the role of this gene family in malignancies is complex, as in synovial cell sarcoma where TLE1 is over-expressed and behaves as an oncogene by pairing with the SS18-SSX fusion oncogene and ATF2 to silence other tumor suppressor genes. Current work in the lab seeks to clarify the role these proteins play in malignancy as well as in normal development. TLE1 and TLE4 are potent inhibitors of the AML1-ETO oncogene in the most common subtype of AML. The mechanism of this inhibition appears to involve both regulation of gene transcription and chromatin structure. In large part this cooperative effect appears to involve regulation of Wnt signaling and



Schematic diagram summarizing proposed TLE4 regulation of AML1-ETO/COX/Wnt axis.

inflammatory gene pathways. This work has led to the demonstration that anti-inflammatory agents can have potent anti-leukemic effects.

Our laboratory is also working to understand the role these proteins play in normal development. To assist in this evaluation, we have generated conditional Tle1 and Tle4 knockout mice and are currently characterizing role these proteins play in the development of a variety of tissues. Our studies to date indicate TLE1 is a potent repressor of inflammation via its ability to repress NFKB, while TLE4 is a critical modulator of neuronal and B-cell and T-cell differentiation, and is required for hematopoietic stem cell maintenance, as well as bone development.

Identification of Novel Inhibitors of AML1-ETO

We have collaborated with the Yeh laboratory to identify several novel small molecule inhibitors of AML1-ETO using a zebrafish high-throughput biological screen. Our results, published in early 2012, identified several classes of agents capable of inhibiting AML1-ETO, and we have demonstrated the efficacy of these agents in treating mouse models of leukemia.

Identifying Genetic Predispositions to Cancer

It is being increasingly recognized that genetic predispositions play a role in the development of many cancers, especially those in children. We are using whole exome sequencing of several cancer types in children to help identify germline mutations that can influence cancer development. Individuals with these mutations may be at higher risk for relapse or the development of additional cancers, and warrant more intensive and extensive surveillance.

The Undiagnosed Diseases Network

The Harvard Medical School hospital consortium of MGH, Brigham and Women's Hospital and Children's Hospital has been recently selected as one of six new sites comprising a nationwide Undiagnosed Diseases Network. As Chief of Medical Genetics at MGH, and the MGH site director for the UDN, Dr. Sweetser is coordinating a team of expert clinicians and researchers, and is using whole exome/whole genome sequencing, paired with RNASeq and metabolomics profiling, and in collaboration with zebrafish and Drosophila model organism cores to identify the underlying basis of a variety of challenging human diseases.

Selected Publications:

Shin TH, Brynczka, Dayyani F, Rivera M, **Sweetser DA**. TLE4 Regulation of Wnt-mediated Inflammation Underlies its Role as a Tumor Suppressor in Myeloid Leukemia. *Leuk Res*. 2016, 48:46-56.

Ramasamy S, Saez B, Mukhopadhyay S, Ding D, Ahemd AM, Chen X, Pucci F, Yamin R, Pittet MJ, Kelleher CM, Scadden DT, **Sweetser DA**. Tle1 tumor suppressor negatively regulates inflammation in vivo and modulates NF-κB inflammatory pathway. *PNAS* 2016, 113:1871-6.

Wheat JC, Krause DS, Shin TH, Chen X, Wang J, Ding D, Yamin R, **Sweetser DA**. The Corepressor Tle4 Is a Novel Regulator of Murine Hematopoiesis and Bone Development. 2014 *PLoS One* 9:e105557

Zhang Y, Wang J, Wheat J, Chen X, Jin S, Sadrzadeh H, Fathi AT, Peterson RT, Kung AL, **Sweetser DA**, Yeh JR. AML1-ETO mediates hematopoietic self-renewal and leukemogenesis through a COX/beta-catenin signaling pathway. *Blood*, 121:4906-4916, 2013.

Yeh JR, Munson K, Alagib K, Goldfarb A, **Sweetser DA,** Peterson RT. Discovering chemical modifiers of oncogene-regulated hematopoietic differentiation. *Nat Chem Biol.* 5:236-243, 2009.

Dayyani F, Wang J, Yeh JR, Ahn EY, Tobey E, Zhang DE, Bernstein ID, Peterson RT, **Sweetser DA**. Loss of TLE1 and TLE4 from the del(9q) commonly deleted region in AML cooperates with AML1-ETO to affect myeloid cell proliferation and survival. *Blood*. 111:4338-4347, 2008.

David Ting, MD

Gastrointestinal cancers are highly lethal cancers where the vast majority of patients are diagnosed too late and conventional therapies have largely been ineffective, making early detection and novel drug targets greatly needed. Recently, a significant amount of "non-coding" repeat RNAs have been found to be produced in high amounts at the earliest stages of cancer development, but not in normal tissues. Interestingly, these satellite RNAs appear to activate a viral response program that appears to help cancers prevent immune cells from attacking them. **The Ting laboratory** has been utilizing innovative microfluidic chip technologies to capture circulating tumor cells (CTCs) and have used satellite RNAs to develop novel blood based early detection biomarkers of cancer. In addition, we are uncovering how these repeat RNAs alter the immune response as a novel immunotherapy target.

Ting Laboratory

Danielle Bestoso
Irun Bhan, MD
Joseph Franses, MD, PhD
Jasmin Joseph
Anupriya Kulkarni, PhD
Matteo Ligorio, MD, PhD
Ann Liu
Neelima Kc Magnus
Tony Nguyen
Julia Philipp, MD, PhD
Rebecca Porter, MD, PhD
Mihir Rajurkar, PhD
Annamaria Szabolcs, MD, PhD
Vishal Thapar, PhD
David T. Ting, MD

The Ting laboratory has utilized RNA-sequencing and RNA in situ hybridization technology to understand the complex transcriptional landscape of cancers. We have used these technologies to characterize non-coding repeat RNA expression across cancer and normal tissues. This has provided novel insight into the role of the repeatome in cancer development and offers a method to identify novel biomarkers and therapeutic targets. In addition, we have been able to capture circulating tumor cells (CTCs) with an innovative microfluidic chip technology and successfully applied RNA-sequencing to these cells to understand their role in the metastatic cascade and to develop novel early detection biomarkers.

Satellite Non-coding RNAs

RNA sequencing of a broad spectrum of carcinomas demonstrated a highly aberrant expression of non-coding satellite RNAs emanating from pericentromeric heterochromatic regions of the genome previously thought to be inactive due to heavy epigenetic silencing. Analysis of all human satellites identified the HSATII satellite as being exquisitely specific for epithelial cancers, including carcinomas of the pancreas, colon, liver, breast, and lung. HSATII expression was confirmed by RNA in situ hybridization (RNA-ISH), and was present in preneoplastic lesions in mouse models and human specimens of the pancreas and colon suggesting satellite expression occurs early in tumorigenesis, which provides for a potential biomarker for early detection and a novel therapeutic avenue. Recently, we have discovered that HSATII is reverse transcribed in cancer cells and can integrate back into the genome and expand these pericentromeric regions. These expansions were found to be a poor prognostic marker in cancer. Moreover, work with others has found that these satellite repeats can affect the local tumor microenvironment with implications for immunotherapies. We are now trying to identify the HSATII reverse transcriptase and better understand the biological role of satellites in cancer progression.

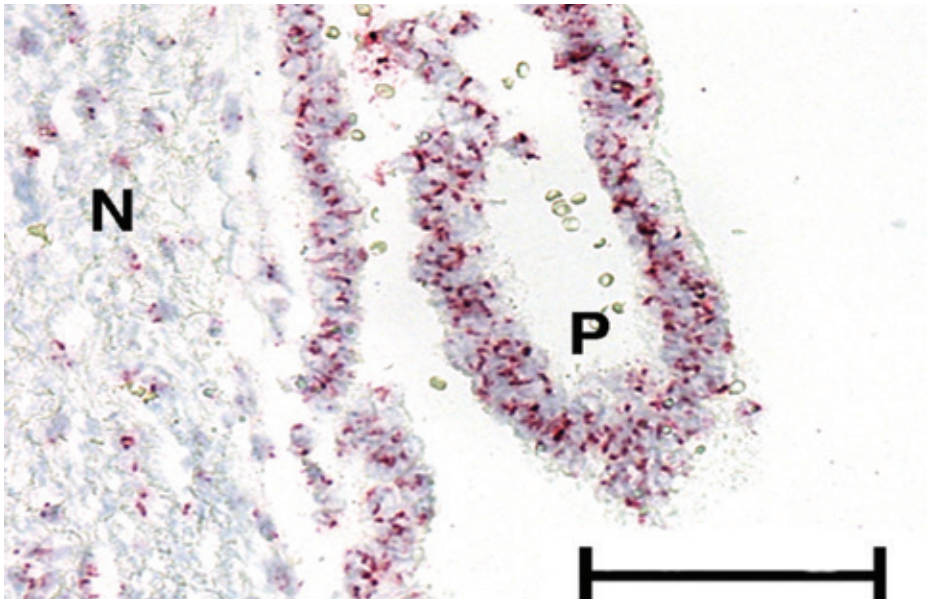


Image of a preneoplastic pancreatic intraepithelial neoplasm (P) positive for the HSATII ncRNA (Red dots).Normal adjacent reactive stroma (N) with minimal expression. Counterstain hematoxylin (blue). Scale bar = 100 μm.

Circulating Tumor Cells: The Liquid Biopsy

The temporal development of circulating tumor cells (CTCs) in tumorigenesis is not well understood, but evidence for CTC shedding in early localized cancers suggests that these cells are heterogeneous and that only a small subset of CTCs have the biological potential to metastasize. Using a novel microfluidic device developed at MGH, we have isolated pancreatic and liver CTCs and perform RNA sequencing on these rare cells. This has revealed the opportunity to develop a novel early detection blood based biomarker and study the metastatic cascade. Using single cell RNA-sequencing, we have characterized the heterogeneity of pancreatic CTCs into three major subclasses, and note that over half of the CTCs are not viable. This illustrates that not all CTCs have the full capacity to metastasize, and that there are likely multiple paths for cancer cell dissemination. In addition, single cell RNA-seq has provided unprecedented transcriptional resolution of CTCs that has revealed significant enrichment for stem cell and epithelial mesenchymal transition markers of these metastatic precursors. Notably, we have also found that CTCs express a significant amount of extracellular matrix proteins normally found in the stroma of primary tumors. This suggests that the seeds of metastasis are in fact producing their own soil during the metastatic cascade. We are currently translating our findings from mouse models to a clinical trial of patients with early pancreatic cancer. The early emergence of CTCs and the opportunity to understand the biology of metastasis in transit offers the potential for developing non-invasive, early detection tools and new strategies to target metastasis.

Selected Publications:

Solovyov A, Vabret N, Arora KS, Snyder A, Funt SA, Bajorin DF, Rosenberg JE, Bhardwaj N, **Ting DT**†, and Greenbaum BD†, Global Cancer Transcriptome Quantifies Repeat Element Polarization between Immunotherapy Responsive and T Cell Suppressive Classes. *Cell Rep*, (2018); 23(2): 512-521.

Desai N*, Sajed D*, Arora KS*, Solovyov A*, Rajurkar M, Bledsoe JR, Sil S, Amri R, Tai E, MacKenzie OC, Mino-Kenudson M, Aryee MJ, Ferrone CR, Berger DL, Rivera MN, Greenbaum BD†, Deshpande V†, **Ting DT**†. Diverse Repetitive Element RNA Expression Define Epigenetic and Immunologic Features of Colon Cancer. *JCI Insight*, (2017); 2(3):e91078.

Bersani F, Lee E, Kharchenko PV, Xu AW, Liu M, Xega K, MacKenzie OC, Brannigan BW, Wittner BS, Jung H, Ramaswamy S, Park PJ, Maheswaran S, **Ting DT**†, and Haber DA†, Pericentromeric satellite repeat expansions through RNA-derived DNA intermediates in cancer. *Proc Natl Acad Sci USA*, (2015); 112(49): 15148-53.

Ting DT*, Lipson D*, Paul S, Brannigan BW, Akhavanfard S, Coffman EJ, Contino G, Deshpande V, Iafrate AJ, Letosky S, Rivera MN, Bardeesy N, Maheswaran S, Haber DA. Aberrant Overexpression of Satellite Repeats in Pancreatic and Other Epithelial Cancers. *Science*, (2011); 331(6017): 593-596. PMCID: PMC3701432

Franses J, Basar O, Kadayifci A, Yuksel O, Choz M, Kulkarni AS, Tai E, Vo KD, Arora KS, Desai N, Licausi JA, Toner M, Maheswaran S, Haber DA, Ryan DP, Brugge WR, **Ting DT**. Improved detection of circulating epithelial cells in patients with intraductal papillary mucinous neoplasms. *The Oncologist* (2017); 22: 1-7.

*Equal contribution

†Co-corresponding

Shobha Vasudevan, PhD

The Vasudevan laboratory focuses on the role of post-transcriptional mechanisms in clinically resistant quiescent cancer cells. Tumors demonstrate heterogeneity, harboring a small subpopulation that switch from rapid proliferation to a specialized, reversibly arrested state of quiescence that decreases their susceptibility to chemotherapy. Quiescent cancer cells resist conventional therapeutics and lead to tumor persistence, resuming cancerous growth upon chemotherapy removal. Our data revealed that post-transcriptional mechanisms are altered, with modification of noncoding RNAs, associated complexes and ribosomes—molecules that control vital genes in cancer—which are important for the persistence of quiescent cancer cells. The primary goal of our research is to characterize the specialized gene expression and their post-transcriptional regulators that underlie persistence of resistant cancer cells. A complementary focus is to investigate the modification of post-transcriptional regulators and their mechanisms in response to quiescent conditions and chemotherapy-induced signaling. Our goal is to develop a comprehensive understanding of the versatile roles of regulatory RNAs in cancer as a basis for early detection of refractory cancers and for designing new therapies.

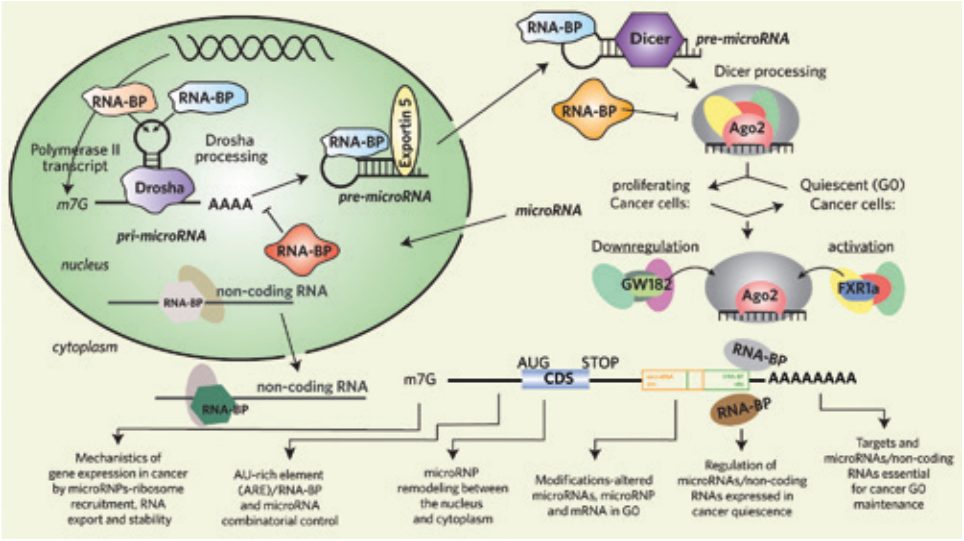
Vasudevan Laboratory

Syed Irfan Bukhari, PhD
Madeleine Granovetter
Jeongmin Lee
Sooncheol Lee, PhD
Yue Lin
Samuel Spencer Truesdell, MEng
Ramzi Elased
Shobha Vasudevan, PhD

Quiescent (G0) cells are observed as a clinically relevant population in leukemias and other tumors associated with poor survival. G0 is a unique, nonproliferative phase that provides an advantageous escape from harsh situations like chemotherapy, allowing cells to evade permanent outcomes of senescence, differentiation, and apoptosis in such tumor-negative environments. Instead, the cell is suspended reversibly in an assortment of transition phases that retain the ability to return to proliferation and contribute to tumor persistence. G0 demonstrates a switch to a distinct gene expression program, upregulating the expression of mRNAs and regulatory non-coding RNAs required for survival. Quiescence regulators that maintain the quiescent, chemoresistant state remain largely undiscovered.

Our studies revealed that specific post-transcriptional regulators, including AU-rich elements (AREs), microRNAs, RNA-protein complexes (RNPs), ribosome factors and RNA modifiers, are directed by G0- and chemotherapy-induced signaling to alter expression of clinically important genes. AU-rich elements (AREs) are conserved mRNA 3'-untranslated region (UTR) elements. MicroRNAs are small noncoding RNAs that target distinct 3'UTR sites. These associate with RNPs, ribosome associated factors and their modifiers to control post-transcriptional expression of cytokines and growth modulators. Their deregulation leads to a wide range of diseases, including tumor growth, immune and developmental disorders.

We identified post-transcriptional effectors associated with mRNAs and noncoding RNAs by developing in vivo crosslinking-coupled RNA affinity purification methods



Regulation of gene expression in cancer by noncoding RNAs and RNPs.

to purify endogenous RNPs. Our recent studies revealed mechanistic changes in G0: uncovering inhibition of conventional translation and its replacement by non-canonical mechanisms that enable specific gene expression in G0 to elicit chemoresistance. These specialized mechanisms are driven by modifications of mRNAs, associated regulator RNAs and proteins, and ribosomes, which are induced in G0- and chemotherapy-induced signaling. These investigations reveal gene expression control by RNA regulators and non-canonical translation mechanisms that cause tumor persistence. Based on our data demonstrating altered RNPs, modifications, and specific translation in G0, we propose that transiently quiescent, chemoresistant subpopulations in cancers are maintained by specialized post-transcriptional mechanisms that permit selective gene expression, necessary for chemotherapy survival and tumor persistence.

The primary goal of our research is to characterize the specialized gene expression program in quiescent, chemoresistant cancers, and its underlying post-transcriptional and translational regulators that contribute to G0 and tumor persistence. A concurrent focus is to investigate RNA modifications and mechanisms of noncoding RNAs, RNPs, and ribosomes in G0 that contribute to chemoresistance, using cancer cell lines, in vivo models, patient samples, and stem cells. An important direction is to identify unique G0-specific RNA markers and develop novel therapeutic approaches to block selective translation in G0, of targets that encode for critical immune and tumor survival regulators—and thereby curtail chemoresistance.

The lab has four core directions:

1. To characterize microRNAs and noncoding RNAs, and their cofactors that control the expression of tumor survival regulators, using in vivo biochemical purification methods.
2. To investigate the mechanisms of post-transcriptional and translational regulation by noncoding RNAs, RNPs, and ribosome regulators.
3. To elucidate the modification and regulation of key mRNAs and ribosomes, by G0- and chemotherapy-induced signaling.
4. To develop therapeutic approaches that interfere with selective translation, and manipulate interactions of noncoding RNAs with targets that encode for critical tumor survival regulators. These studies should lead to a greater understanding of the versatile role of post-transcriptional mechanisms in cancer persistence and to novel approaches in RNA-based therapeutics.

Selected Publications:

Bukhari SI, and **Vasudevan S**. FXR1-associated microRNP: A driver of specialized, non-canonical translation in quiescent conditions. *RNA Biology*. 2017; 14(2):137-145. doi: 10.1080/15476286.2016.1265197.

Martinez I, Hayes K, Barr J, Harold A, Xie M, Bukhari SIA, **Vasudevan S**, Steit, JA, DiMaio D. An exportin-1 dependent microRNA biogenesis pathway during human cell quiescence. *PNAS*. 2017; 114(25):E4961-E4970. doi:10.1073/pnas.1618732114

Bukhari SI, Truesdell SS, J Lee S, Kollu S, Classon A, Boukhali M, Jain E, Mortensen RD, Yanagiya A, Sadreyev RI, Haas W, and **Vasudevan S**. A specialized mechanism of translation mediated by FXR1a-associated microRNP in cellular quiescence. *Molecular Cell*. 2016; 61(5):760-773.

Lee S, Truesdell SS, Bukhari SIA, Lee JH, LeTonqueze O and **Vasudevan S**. Upregulation of eIF5B controls cell cycle arrest and specific developmental stages. *PNAS*. 2014; 111(41):E4315-22.

Liu M, Roth A, Yu M, Morris R, Bersani F, Rivera MN, Lu J, Shioda T, **Vasudevan S**, Ramaswamy S, Maheswaran S, Diederichs S, Haber DA. The IGF2 intronic miR-483 selectively enhances transcription from IGF2 fetal promoters and enhances tumorigenesis. *Genes & Dev*. 2013; 27(23):2543-8.

Chen A-J, Paik J-H, Zhang H, Shukla SA, Mortensen RD, Hu J, Ying H, Hu B, Hurt J, Farny N, Dong C, Xiao Y, Wang YA, Silver PA, Chin L, **Vasudevan S** and DePinho RA. Star RNA-binding protein, Quaking, suppresses cancer via stabilization of specific miRNA. *Genes Dev*. 2012; 26(13):1459-72.

Alexandra-Chloé Villani, PhD

The Villani laboratory seeks to establish a comprehensive roadmap of the human immune system by achieving a higher resolution definition and functional characterization of cell subsets and rules governing immune response regulation, as a foundation to decipher how immunity is dysregulated in diseases. We use unbiased systems immunology approaches, cutting-edge immunogenomics, single-cell 'multi-omics' strategies, and integrative computational frameworks to empower the study and modeling of the immune system as a function of "healthy" and inflammatory states, disease progression, and response to treatment. Our multi-disciplinary team of immunologists, geneticist, computational biologists, and physicians work towards answering several key questions: Do we know all existing blood immune cell subsets? How do circulating immune cells mirror those in tissue microenvironment in the context of health and disease? Can we identify targets that would improve immunotherapy efficacy by increasing specificity? Collectively, our groundwork is paving the way for developing a human immune lexicon that is key to promoting effective bench-to-beside translation of findings.

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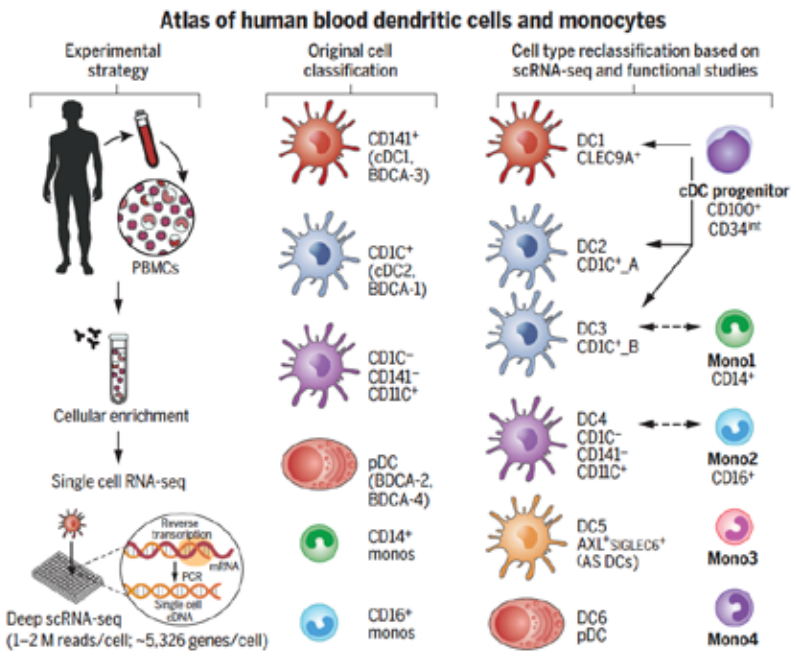
Villani Laboratory

Molly Fisher Thomas, MD, PhD
Mazen Nasrallah, MD, MSc
Daniel Zlotoff, MD, PhD
Kasidet Manakong treecheep, BSc

Leveraging single-cell '*omics*' to unravel new insights into human immune system

Achieving detailed understanding of the composition and function of the immune system at the fundamental unit of life — the cell — is essential to determining the prerequisites of health and disease. Historically, leukocyte populations have been defined by a combination of morphology, localization, functions, developmental origins, and the expression of a restricted set of markers. These strategies are inherently biased and recognized today as inadequate. Single-cell RNA sequencing (scRNAseq) analysis provides an unbiased, data-driven way of systematically detecting cellular states that can reveal diverse simultaneous facets of cellular identity, from discrete cell types to continuous dynamic transitions, which cannot be defined by a handful of pre-defined markers or for which markers are not yet known. We combine scRNAseq strategies together with in-depth follow-up profiling, phenotypic and functional characterization of prospectively isolated immune subsets defined by scRNAseq data to overcome such limitations. Our analyses of the human blood mononuclear phagocyte system resulted in the identification of six dendritic cell (DC), four monocyte, and one DC progenitor populations, thus revising the taxonomy of these cells (*Villani et al., Science 2017*). Noteworthy, five of these subsets had never been reported, illustrating the power of our integrative strategies to reopen the definition of these cell types. Our study highlighted the value of embarking on a comprehensive Human Cell Atlas initiative and offered a useful framework for conducting this kind of analysis on other cell types and tissues. We are currently contributing to the immune



Establishing a human blood dendritic cell and monocyte atlas. *We isolated ~2400 cells enriched from the healthy human blood lineage− HLA-DR+ compartment and subjected them to single-cell RNA sequencing. This strategy, together with follow-up profiling and functional and phenotypic characterization, led us to update the original cell classification to include six DCs, four monocyte subtypes, and one conventional DC progenitor.*

cell atlas effort by charting at high-resolution the human blood cellular landscape, and are studying paired human tissues with blood to better establish how circulating immune cells mirror those in tissue microenvironment in the context of health and disease.

We also continuously support development of in-depth expertise in single-cell 'omics' approaches, including single-cells strategies to map X-chromosome inactivation (*Tukiainen, Villani et al., Nature 2017*), new enrichment method targeting individual cell transcriptome in pooled library (*Ranu, Villani et al., Nucleic Acid Res 2019*), method's development to study single-T cells (*Villani et al., Methods Mol Biol 2016*) and application to study T cells infiltrates in melanoma lesions (*Izar, et al., Science 2016*).

Deciphering immune-related adverse events (irAEs) induced by immune-checkpoint inhibitor (ICI) therapy.

While ICI therapy is revolutionizing the treatment of solid cancers, its success is currently being limited by treatment-induced irAEs resembling autoimmune diseases that are affecting nearly every organ system. With ICI becoming first- and second-line of cancer treatments, it is expected that the number of irAEs will continue rising and limit immunotherapy efficacy unless we find solutions. Our multi-disciplinary translational group of scientists and clinicians are working towards developing a better understanding of the biological players and underlying molecular and cellular mechanisms involved in driving irAEs by directly studying patient blood and matched affected tissue samples using a range of systems immunology, immunogenomics and single-cell 'omics' strategies. Our translational research program may result in identifying putative cellular components and mechanisms that could be (i) targeted in a 'primary-prevention' approach to prevent irAE development, or (ii)targeted after onset of irAEs, without reducing the efficacy of the immunotherapy.

Selected Publications:

Villani AC, Sarkizova S, Hacohen N. Systems Immunology: learning the rules of the immune system. *Annu Rev Immunol* 2018; 36: 813-842.

Villani AC*†, Satija R*, Reynolds G, Sarkizova S, Shekhar K, Fletcher J, Griesbeck M, Butler A, Zheng S, Lazo S, Jardine L, Dixon D, Stephenson E, Nilsson E, Grundberg I, McDonald D, Filby A, Li W, De Jager PL, Rozenblatt-Rosen O, Lane AA, Haniffa M, Regev A†, Hacohen N†. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes and progenitors. *Science* 2017; 356: 6335. pii: eaah4573.

Tukiainen T, **Villani AC**, Yen A, Rivas MA, Marshall JL, Satija R, Aguirre M,Gauthier L, Fleharty M, Kirby A, Cummings BB, Castel SE, Karczewski KJ, Aguet F, Byrnes A, GTEx Consortium, Lappalainen T, Regev A, Ardlie KG, Hacohen N, MacArthur DG. Landscape of X chromosome inactivation across human tissues. *Nature* 2017; 550(7675): 244-248.

Ranu N, **Villani AC**, Hacohen N, Blainey PC. Targeting individual cells by barcode in pooled sequence library. *Biorxiv* 2017; doi: https://doi.org/10.1101/178681.

Villani AC†, Karthik Shekhar†. Single cell RNA sequencing of human T cells. *Methods in Molecular Biology* 2017; 1514: 203-239.

Olah M*, Patrick E*, **Villani AC***, Xu J, White CC, Ryan KJ, Piehowski P, Kapasi A, Nejad P, Cimpean M, Connor S, Yung CJ, Frangieh M, McHenry A, Elyaman W, Petyuk V, Schneider JA, Bennett DA, De Jager PL, Brashaw EM. A transcriptomic atlas of aged human microglia informs neurodegenerative disease studies. *Nat Communications* 2018; 9(1): 539.

Johnathan R. Whetstine, PhD

The Whetstine laboratory is interested in understanding how the chromatin microenvironment regulates gene expression while maintaining a stable genome. Our ultimate goal is to harness this mechanistic understanding to identify novel therapeutic opportunities and to block chemotherapeutic resistance. We integrate biochemistry, genetics, genomics and computation to elucidate chromatin modulators involved in these processes. We have initiated these types of studies by focusing on a specific class of chromatin regulators, the JmjC-containing histone demethylases and histone lysine methyltransferases. My laboratory screens tumors for genomic anomalies (copy changes and mutations) in these classes of enzymes and examines their molecular roles at a biochemical, molecular and in vivo level. These combined approaches will determine whether tumors with alterations in JmjC enzymes or lysine methyltransferases provide an opportunity to modify conventional chemotherapy, to uncover mechanisms of drug resistance, and to identify novel molecular diagnostics.

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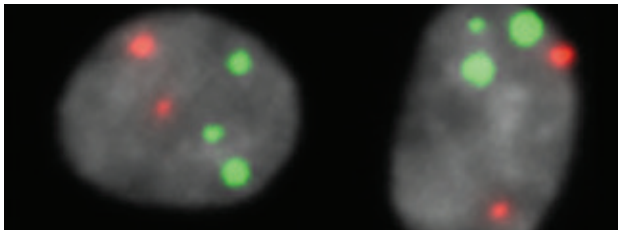
Whetstine Laboratory

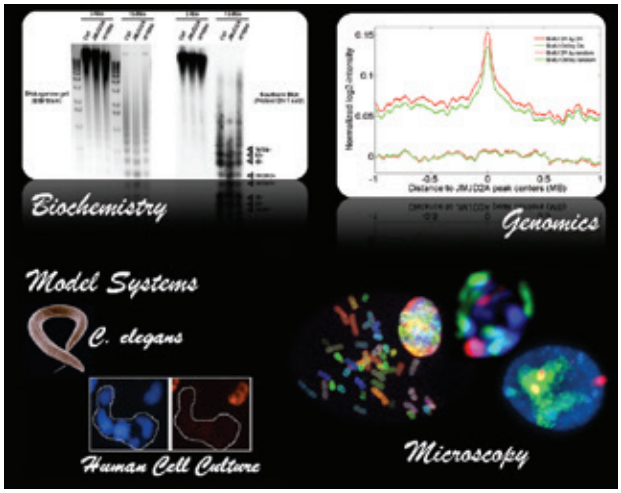
Damayanti Chakraborty, PhD
Thomas Clarke, PhD
Sweta Mishra, PhD
Capucine Van Rechem, PhD
Johnathan R. Whetstine, PhD

Understanding How Epigenetics Directly Impacts Cancer Progression and Drug Response

The N-terminal tails of histones are subject to a plethora of post translational modifications (PTMs). Each modification can affect chromatin architecture, but the sum of these modifications may be the ultimate determinant of the chromatin state and biological outcome. Research has shown that multiple lysine (K) residues on the tails of histone H3 and H4 are sites for methylation. The site and degree of methylation (mono-, di-, or tri-) are linked to transcriptional activation and repression, cell cycle progression, and DNA damage response. Heterochromatin formation and X-inactivation are regulated by histone methylation; therefore, aberrant methylation can result in human diseases such as cancer. For this reason, organisms have enzymes that are responsible for both adding and removing the methyl marks.

My laboratory investigates how the interplay between the enzymes that are adding lysine methylation (referred to as lysine methyltransferases, KMTs) and removing the methylation states (referred to as lysine demethylases, KDMs) impacts transcriptional and post transcriptional regulation of the genome [Van Rechem et al. (2015) *Cancer Discovery* and Black et al. (2016) *J. Biol. Chem.*)], cell cycle progression through altering chromatin organization [Black et al. (2010) *Mol. Cell* and Van Rechem et al. (2011) *J. Biol. Chem.*], and genome stability (i.e., DNA amplifications and rearrangements) [Black et al. (2013) *Cell*, Black et al. (2015) *Genes and Development*, Black et al. (2016) *J. Biol. Chem.*]. In fact, our group was the first to discover that lysine modifying enzymes and their associated methylate states are critical modulators of site-specific rereplication and DNA amplification of regions promoting drug resistance through their associated gene products [Black et al. (2013) *Cell*] (upper





The upper image illustrates the site-specific DNA copy gains that occur upon overexpression or stabilization of the histone 3 lysine 9/36 tri-demethylase KDM4A. The nuclei are white and the genomic regions that undergo copy gains are green, while regions not impacted by KDM4A overexpression or stabilization are in red. Data related to this image are in Black et al (2015) Genes and Development.

The lower image represents the types of approaches that the Whetstine laboratory is using to understand the impact that chromatin and the associated modulatory factors have on development and cancer.

image). These studies have opened an entirely new concept around the modulation of DNA amplification and how these events can occur across and within tumors. We are actively expanding these studies within the group.

While resolving the molecular roles these enzymes play in cancer, we are also uncovering the physiological pathways that directly modulate the activity, stability and function of these enzymes. For example, these studies have allowed us to better understand how types of cell stress or signaling events impact epigenetic regulators, and in turn, gene expression, DNA amplification and drug response within tumors [Black et al. (2015) *Genes and Development*]. Understanding the epigenetic mechanisms that influence gene expression and genomic heterogeneity in tumors will allow biomarkers and drug targets to be identified in order to circumvent this major challenge in treating cancer from pediatrics to adults and hematological to solid cancers. This area is of significant interest to the laboratory.

The laboratory uses a range of approaches to interrogate the functional role epigenetic regulators: genomic (ChIP-seq and RNA-seq), proteomic (MS-MS complexes and PTMs), cytologic (live imaging and deconvolution confocal microscopy) and genetic (human cell models and models systems) (lower panel). Using these strategies, we have uncovered a conserved role for JMJD2A in genomic stability and DNA replication [Black et al. (2010) *Mol. Cell* and Van Rechem et al. (2011) *JBC*]. Furthermore, we uncovered a conserved role for chromatin states and KDM4A in modulating rereplication at specific sites in the genome. The rereplication promotes site-specific copy gains of drug resistant regions in both human and zebrafish cells [Black et al. (2015) *Genes and Development*]. This series of discoveries identified the first enzyme, physiological condition and chromatin states that modulate copy gain and selection of drug resistant regions across cancer types. Therefore, combining model systems with human cell culture models as well as integrating multiple approaches, we are poised to uncovered mechanisms impacting genome stability and drug resistant gene selection across tumors (lower image).

Selected Publications:

Black JC, Zhang H, Kim J, Getz G, **Whetstine JR**. Regulation of transient site-specific gain by microRNA. *J. Biol. Chem.* 2016; 291, 4862-4871.

Black JC, Atabakhsh E, Kim J, Biette KB, Van Rechem C, Ladd B, Burrowes Pd, Donado C, Mattoo H, Kleinstiver BP, Song B, Andriani G, Joung JK, Iliopoulos O, Montagna C, Pillai S, Getz G, **Whetstine JR**. Hypoxia drives transient site-specific copy gain and drug-resistant gene expression. *Genes and Development.* 2015; 29, 1018- 1031.

Van Rechem C, Black JC, Greninger P, Zhao Y, Donado C, Burrowes Pd, Ladd, B, Christiani DC, Benes CH, **Whetstine JR**. A Coding Single Nucleotide Polymorphism in Lysine Demethylase KDM4A Associates with Increased Sensitivity to mTOR Inhibitors. *Cancer Discov.* 2015; 5, 245-254.

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Black JC, Allen A, Van Rechem C, Forbes E, Longworth M, Tschöp K, Rinehart C, Quiton J, Walsh R, Smallwood A, Dyson NJ, **Whetstine JR**. Conserved antagonism between JMJD2A/KDM4A and HP1 during cell cycle progression. *Mol Cell*. 2010 Dec 10; 40(5):736-48.

Lee Zou, PhD

Cancer is a complex disease driven by genetic and epigenetic alterations in the genome. To prevent these detrimental alterations, cells have evolved an intricate signaling network, called the checkpoint, to detect and signal problems in the genome. During cancer development, the activation of oncogenes and loss of tumor suppressors leads to genomic instability, rendering cancer cells increasingly dependent upon specific DNA repair and checkpoint signaling proteins to survive. **The Zou laboratory** is particularly interested in understanding how the checkpoint detects DNA damage and genomic instability, and how the checkpoint can be targeted in cancer therapy. Our current studies are focused on the activation of ATR and ATM, the master sensor kinases of two major checkpoint pathways. Furthermore, we are developing new strategies to exploit the genomic instability and checkpoint addiction of different cancer cells in targeted cancer therapy.

Zou Laboratory

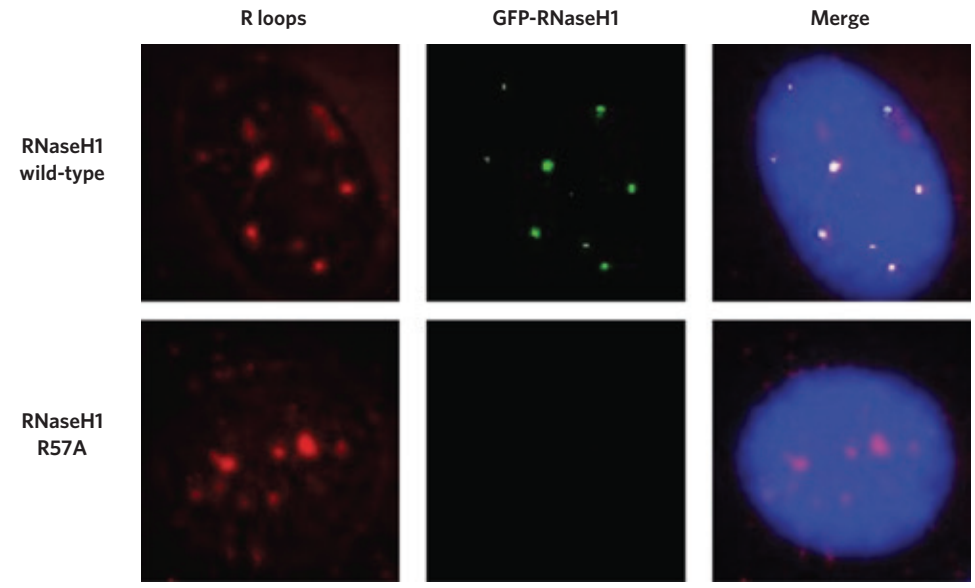
Remi Buisson, PhD
Marie-Michelle Genois, PhD
Lilian Kabeche, PhD
Dominick Matos
David Moquin, PhD
Hai Dang Nguyen, PhD
Jian Ouyang, PhD
Antoine Simoneau, PhD
Jack Sullivan
Tribhuwan Yadav, PhD
Takaaki Yasuhara, PhD
Jiamin Zhang, PhD
Lee Zou, PhD

Sensing of DNA Damage, Replication Stress, and Transcription Problems

ATM and ATR are two master checkpoint kinases in human cells. In particular, ATR is the key responder to a broad spectrum of DNA damage and DNA replication problems. To understand how ATR is activated, we sought to identify the key DNA structural elements and sensor proteins that activate ATR. We have developed unique biochemical and cell biological assays to dissect the process of ATR activation. Our recent studies have revealed that ATR is not only important for sensing DNA damage and replication stress, but also for problems associated with transcription. R loops, which arise from stable DNA:RNA hybrids during transcription, are a major source of genomic instability. We found that ATR is activated by R loops and plays a key role in suppressing R loop-induced genomic instability, thus, uncovering a new function of ATR in safeguarding the genome.

Checkpoint, DNA Replication, DNA Repair, Telomeres, Centromeres and the Cell Cycle

The ATR checkpoint plays a key role in regulating and coordinating DNA replication, DNA repair, and cell cycle transitions. During the past few years, our studies have identified a number of novel roles that ATR plays in protecting the genome, such as: suppressing single-stranded DNA (ssDNA) accumulation during DNA replication, regulating homologous recombination (HR), and promoting alternative lengthening of telomeres (ALT). This year, we have discovered a surprising function of ATR in mitosis. We have shown that ATR is localized to centromeres in mitosis, where it is activated by centromeric R loops. The activation of ATR at centromeres is critical for faithful chromosome segregation, thus revealing the unexpected importance of ATR in suppressing chromosomal instability (CIN).



This image shows that GFP-tagged RNaseH1 (green) localizes to sites of R loops (red) through binding to RPA. R loops are transcription intermediates that contain RNA:DNA hybrids and single-stranded DNA (ssDNA). RPA is a protein complex that recognizes ssDNA. RNaseH1 is an enzyme that suppresses R loops by cleaving the RNA in RNA:DNA hybrids. Wild-type RNaseH1 recognizes R loops through binding to RPA, but the R57A mutant of RNaseH1, which is defective for RPA binding, fails to recognize R loops.

Checkpoint Signaling, Non-Coding RNA, and Epigenetic Regulation

The signaling of DNA damage through the checkpoint pathway is generally viewed as a cascade of protein phosphorylation events. However, recent studies by us and others have revealed that many types of modifications of proteins and chromatin—such as ubiquitylation, SUMOylation, methylation and acetylation—also contribute to DNA damage signaling. Furthermore, noncoding RNAs have also been implicated in this process. We are currently investigating how this network of regulatory events is integrated to the DNA damage response.

Checkpoint Inhibitors and Targeted Cancer Therapy

While the checkpoint is often compromised in cancers, certain checkpoint proteins are uniquely required for the survival of cancer cells because of the oncogenic events within them. We recently discovered that APOBEC3A/B proteins, two cytidine deaminases that are aberrantly expressed in multiple types of cancers, induce DNA replication stress and render cancer cells susceptible to ATR inhibition. Furthermore, the splicing factor mutants found in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) induce R loops and trigger an ATR response. Cells that express these splicing factor mutants are sensitive to ATR inhibitors, providing a new strategy for the treatment of MDS and possibly other malignancies associated with RNA splicing defects.

Selected Publications:

Nguyen, HD, Leong, WY, Li W, Walter M, **Zou L**, and Graubert T. (2018) Spliceosome mutations in myelodysplastic syndrome induce R loop-associated sensitivity to ATR inhibition. *Cancer Res.* (July 27, 2018; Epub ahead of print.)

Kabeche L, Nguyen HD, Buisson R, and **Zou L**. (2018) A mitosis-specific and R loop-driven ATR pathway promotes faithful chromosome segregation. *Science* 359:108-114.

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Flynn RL, Centore RC, O'Sullivan RJ, Rai R, Tse A, Songyang Z, Chang S, Karlseder J, **Zou L**. (2011) TERRA and hnRNPA1 orchestrate an RPA-to-POT1 switch on telomeric single-stranded DNA. *Nature*. 471:532-6.

MASSACHUSETTS
GENERAL HOSPITAL

CANCER CENTER

CENTER FOR CANCER RESEARCH

Charlestown Laboratories
Building 149, 13th Street
Charlestown, MA 02129

Jackson Laboratories
Jackson Building
55 Fruit Street
Boston, MA 02114

Simches Laboratories
CPZN 4200
185 Cambridge Street
Boston, MA 02114

www.massgeneral.org/cancerresearch/