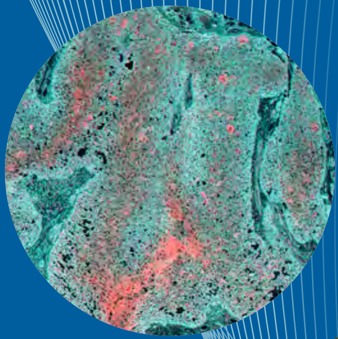
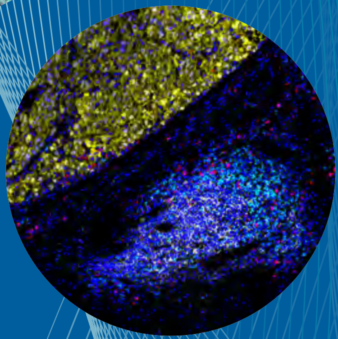
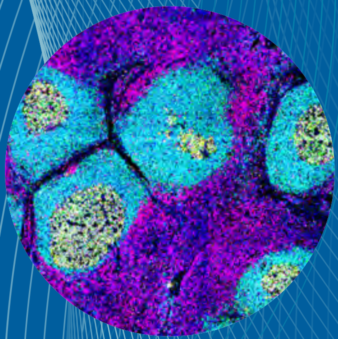
Mass General Brigham
Mass General Cancer Center

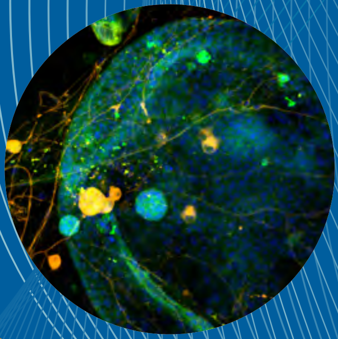


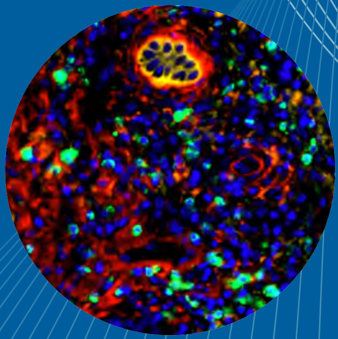


KRANTZ FAMILY Center for Cancer Research

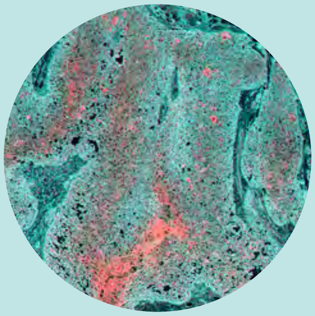


Annual Report 2023-2024



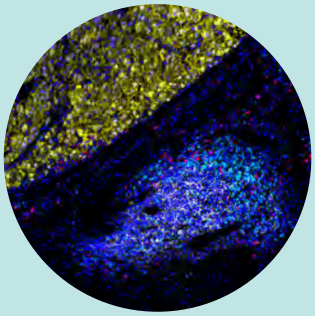


Featured images from front cover



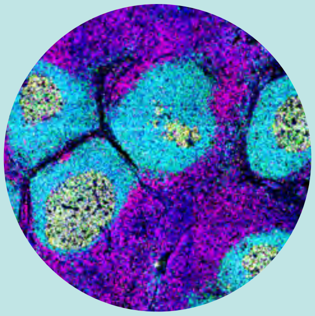
Damage-associated molecular patterns (red) expression in skin cancer.

Image courtesy of Marjan Azin, MD, the Demehri Laboratory



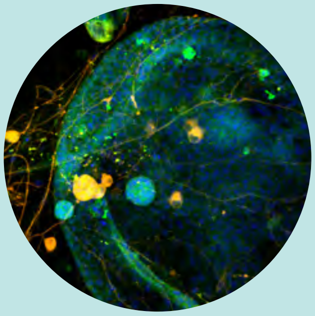
Immune bastion ready to attack melanoma: Immune cells forming an ectopic follicle-like structure composed of B cells (CD19-light grey), CD8 T cells (CD8a-red), CD4 T cells (CD4-cyan) infiltrating or in the vicinity of Melanoma tumors (S100-yellow). Additional markers: pan-Cytokeratin (Pan CK-pink) and cell nuclei (blue).

Image Courtesy of the Lloyd Bod laboratory



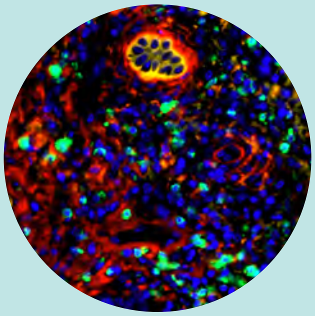
Early loss of germinal centers and Bcl-6 expressing B cells in COVID-19 thoracic lymph nodes. Overlay of low-power images of CD3 (red), CD19 (green), Bcl-6 (orange) and DAPI (blue) staining in a lymph node from a late COVID-19 patient and control.

Image courtesy of Naoki Kaneko, DDS, PhD, the Pillai Laboratory



Cancer-Nerve Tumoroids: A new 3D method of growing genetically-engineered cancer cells (green) together with nerves (orange) in a tissue matrix that mimics the tumor microenvironment.

Image courtesy of the William Hwang lab



Regulation of NK cells by extracellular matrix proteins: Natural killer (NK) cells (green) are embedded in the collagen (red) matrix as they try to reach their target (mutant keratinocytes, yellow) in the skin.

Image courtesy of the Shawn Demehri lab

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Director's Message

It is with great excitement, joy and deep appreciation that we launch a new era in cancer research at Mass General Cancer Center with the naming of the **Krantz Family Center for Cancer Research**. Our goal is to transform an already exceptional research program into a preeminent cancer research center that aims to solve the toughest scientific challenges in cancer, to the great benefit of all patients with cancer and their families.

The Center for Cancer Research (CCR) has served as the hub for basic and translational research within the Mass General Cancer Center since its inception in 1988. It has been the "engine for discovery," and the home to over 50 extraordinary faculty and over 500 researchers, including students, postdoctoral scientists, research scientists and technologists, committed both to fundamental discovery and to its application in cancer. Our research laboratories focus on subjects from the fundamental biology of cellular proliferation to the molecular analysis of patient-derived clinical specimens, and from the creation of novel drug and immune therapies to the integration of algorithms based on artificial intelligence for high-throughput data analysis. We are particularly proud of the ingrained culture of multidisciplinary collaboration across different laboratories and between basic scientists and clinical researchers.

The September 2023 opening of the Krantz Family Center for Cancer Research will greatly expand and accelerate our research to enhance its success and impact. Thanks to the incomparable generosity of Jason and Keely Krantz, we are launching multiple annual Breakthrough and Quantum Awards, along with Pilot and Advanced Technology Grants, that will enable our researchers to work together in boldly taking on major challenges with the resources required for transformative success. We aim to uncover new scientific insights into the biological drivers and vulnerabilities of cancer; design novel approaches to detecting early, curable cancers; develop powerful, scientifically driven, rational therapies; and understand the complex interactions between tumor cells and their surrounding immune microenvironment to suppress the growth and spread of cancer. Together, the Krantz Family Awards will fuel a wave of progress and discovery, and leverage multidisciplinary, team-driven "convergent science," with the ultimate goal of transforming the care of patients with cancer.

We are pleased to share with you our Annual Scientific Report for 2023-2024. Thank you for your interest in our mission of discovery and innovation.

Daniel A. Haber, MD, PhD
Director, Krantz Family Center for Cancer Research

Daniel Haber photo by Scott Eisen

The Krantz Family Center for Cancer Research

Inspired by the vision, creativity, care and leadership
that define the spirit of the Mass General Cancer Center,

JASON R. AND KEELY F. KRANTZ

are honored to name the

KRANTZ FAMILY
CENTER FOR CANCER RESEARCH

With the enduring intent that this philanthropic endeavor
will pioneer impactful advances in cancer detection,
treatment and prevention, and enable scientists to launch bold
and innovative research to vanquish this disease.

Jason and Keely Krantz photo by Austin Wilder/Cataumet Creative

About the Krantz Family Center for Cancer Research

The Krantz Family Center for Cancer Research includes over 50 Principal Investigators with Harvard Medical School (HMS) appointments in the Departments of Medicine, Pathology, Radiation Oncology, Surgery, Dermatology and Pediatrics, as well as at the Broad Institute of MIT and Harvard. Together with over 500 investigators, they conduct research in 80,000 square feet of laboratory space in three Massachusetts General Hospital research facilities: Charlestown Navy Yard, the Simches Research Building and the Jackson Building. Ongoing research projects explore cancer genetics, genomics, epigenetics and proteomics, developmental biology, cell signaling, cancer diagnostics, molecular therapeutics and drug resistance, immunology and immunotherapy, cellular metabolism, cell cycle regulation, RNA biology, and computational biology.

Since the creation of the Mass General Cancer Center in 1988, landmark publications from our faculty have included the first discovery of TP53 germline mutations conferring familial susceptibility to cancer (Malkin, et al., *Science*, 1990) and the major contribution of "founder BRCA1 mutations" to early-onset breast cancer in Ashkenazi populations (FitzGerald, et al., *New England Journal of Medicine*, 1996). Our investigators first cloned the E2F gene, the primary cell cycle regulator that is unleashed by cancer-associated mutations in the RB1 retinoblastoma tumor suppressor (Helin, et al., *Cell*, 1992). Using functional screens in fruit fly genetic models, scientists first discovered the Fbxw7/Ago (Moberg, et al., *Nature*, 2001) and Hippo/YAP (Harvey, et al., *Cell*, 2003) pathways, major drivers of cancer proliferation.

In 2004, researchers identified activating mutations in the *EGFR* gene, which drive 10% of all lung cancers and underlie their extreme sensitivity to targeted kinase inhibitors (Lynch, et al., *New England Journal of Medicine*, 2004). This discovery helped launch the field of "precision oncology" in solid tumors; it set in motion major initiatives in molecular genotyping of cancers to guide therapy and the application of accelerated early phase clinical trials of targeted therapies for genotyped cancers. Mass General was the first hospital in the U.S. to establish genotyping as part of standard clinical care for cancer in 2008, and in 2011, the Cancer Center launched the Termeer Center for Targeted Therapies, which has emerged as an internationally renowned center of excellence for first-in-human clinical trials. It is through this integration of transformative research and exceptional clinical care that the Mass General Cancer Center has emerged internationally as a recognized leader in cancer research and innovation.

Today, our investigators continue to actively pursue fundamental questions in cancer biology, together with translational applications with potential clinical impact. Major areas of emphasis include our *Center for Molecular Therapeutics*, bringing together high-throughput cellular screens, proteome-wide targeting of reactive cysteines, metabolomics-directed drug targets and unique patient-derived tumor models; *Circulating Tumor Cell Biology*, a unique partnership between bioengineers, molecular biologists and clinicians to create tools and develop insights into blood-based spread of cancer; *CAR-T & Cellular Immunotherapy*, a rapidly expanding program to design novel cellular therapies from initial concept through to first-in-human clinical trials; *Cancer Immunology*, a comprehensive research program that includes topics from single-cell and spatial transcriptomic mapping of patient-derived biopsies to creation of new-generation cancer vaccines; *Rare Cancer Initiatives*, a focus on cancers with specific features that are understudied yet potentially treatable; and *Advanced Proteomics & Computational Biology*, an initiative combining next-generation mass spectrometric analytics of proteins in blood and human tissues with machine-learning algorithms, transforming their capabilities and applications. Beyond these highlights, all Krantz Center faculty pursue their scientific vision, as detailed in the individual reports of Principal Investigators in this Annual Report.

The Krantz Center greatly values creativity and innovation across multiple disciplines of cancer research, and we are proud of our strong culture of collaboration and collegiality, demonstrated by multiple co-authored manuscripts, joint laboratory meetings and cross-laboratory team science. We are committed to increasing diversity among our faculty and trainees and to directing scientific discovery toward areas of unmet need in our society. Finally, it is through training and mentoring the next generation of young scientists that we will continue to harness the power of science and uncover new and more effective ways to fight cancer.

Scientific Advisory Board

2023–2024 Members

Dafna Bar-Sagi, PhD
NYU Langone Health

David E. Fisher, MD, PhD
Massachusetts General Hospital

Robert E. Kingston, PhD
Massachusetts General Hospital

David N. Louis, MD
Massachusetts General Hospital

M. Celeste Simon, PhD
The Abramson Family Cancer Research Institute
University of Pennsylvania Perelman School of Medicine

Past Members

Julian Adams
Gamida Cell, Ltd

Spyros Artavanis-Tsakonas, PhD
Harvard Medical School

Joseph Avruch, MD
Massachusetts General Hospital

David Baltimore, PhD
Broad Institute

Cori Bargmann, PhD
Rockefeller University

Edward J. Benz Jr., MD
President Emeritus, *Dana-Farber Cancer Institute*
Candel Therapeutics

Joan S. Brugge, PhD
Harvard Ludwig Cancer Center

Donald Ganem, MD
University of California, San Francisco

Walter J. Gehring, PhD🎗
Biozentrum
University of Basel

Richard O. Hynes, PhD
Massachusetts Institute of Technology

David Hogness, PhD🎗
Stanford University School of Medicine

David Housman, PhD
Massachusetts Institute of Technology

Peter Howley, MD
Harvard Medical School

Tyler Jacks, PhD
Massachusetts Institute of Technology
Founding Director, Koch Institute for Integrative Cancer Research at MIT

Alfred G. Knudson Jr., MD, PhD🎗
Fox Chase Cancer Center

David Livingston, MD🎗
Dana-Farber Cancer Institute

Scott Lowe, PhD
Memorial Sloan Kettering Cancer Center

Frank McCormick, PhD
University of California, San Francisco

Stuart Orkin, MD
Children's Hospital and
Dana-Farber Cancer Institute

Terry Orr-Weaver, PhD
Professor Emerita, Whitehead Institute

Anthony Pawson, FRS, PhD
Samuel Lunenfeld Research Institute,
Mount Sinai Hospital

Carol Prives, PhD
Columbia University

Gerald M. Rubin, PhD
University of California, Berkeley

Gary Ruvkun, PhD
Massachusetts General Hospital

Jeffrey Settleman, PhD
Pfizer, Inc.

Phillip A. Sharp, PhD
Massachusetts Institute of Technology

Arlene Sharpe, MD, PhD
Harvard Medical School

Eileen White, PhD
Rutgers University Cancer Institute of New Jersey

🎗*In Memoriam*

The Jonathan Kraft Prize for Excellence in Cancer Research

Presented by the Mass General Cancer Center

2024
Howard Y. Chang, MD, PhD
Virginia and D.K. Ludwig Professor of Cancer Genomics
Professor of Dermatology and of Genetics
Stanford University School of Medicine

2023
Michelle Monje, MD, PhD
Professor of Neurology
Stanford University School of Medicine

2021
Aviv Regev, PhD
Head, Genentech Research and Early Development
Core Member (on leave), Broad Institute of Harvard and MIT
Professor of Biology, MIT

2019
Carl H. June, MD
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

🎗 *In Memoriam*

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

Faculty

Krantz Family Center for Cancer Research Faculty

Leadership

Daniel A. Haber, MD, PhD
Director, Krantz Family Center for Cancer Research
Director, Mass General Cancer Center
Kurt J. Isselbacher Professor of Oncology (Medicine)
Investigator, Howard Hughes Medical Institute

Raul Mostoslavsky, MD, PhD
Scientific Director, Krantz Family Center for Cancer Research
Laurel Schwartz Professor in Medicine in the Field of Oncology
Professor of Medicine

Andrea I. McClatchey, PhD
Director for Academic Affairs, Krantz Family Center for Cancer Research
Poitras Family Endowed Chair in Oncology
Professor of Pathology

Nir Hacohen, PhD
Director, Center for Cancer Immunology, Krantz Family Center for Cancer Research
Director, Center for Cell Circuits, Broad Institute of Harvard and MIT
David P. Ryan Endowed Chair in Cancer Research
Professor of Medicine

Charlestown Laboratories

Liron Bar-Peled, PhD
Assistant Professor of Medicine

Lloyd Bod, PhD
Assistant Professor of Medicine

Ryan B. Corcoran, MD, PhD
Director, Cancer Center-Tucker Gosnell Center for Gastrointestinal Cancers
Mark J. Kusek Endowed Chair in Colorectal Cancer
Associate Professor of Medicine

Shawn Demehri, MD, PhD
MGH Research Scholar 2023-2028
Associate Professor in Dermatology (Cutaneous Biology Research Center)

Andrew Elia MD, PhD
Assistant Professor of Radiation Oncology

David E. Fisher, MD, PhD
Director, Cancer Center Melanoma Program
Director, Cutaneous Biology Research Center
Lancer Professor of Dermatology
Edward Wigglesworth Professor and Chair of Dermatology

Gaddy Getz, PhD
Director of Bioinformatics, Cancer Center and Pathology
Director of Cancer Bioinformatics, Broad Institute of Harvard and MIT
Paul Zamecnik, MD Endowed Chair in Oncology Basic Research
Professor of Pathology

Francesca Gazzaniga, PhD
Assistant Professor of Pathology (Molecular Pathology Unit)

Doğa C. Gülhan, PhD
*Faculty Member**

Timothy A. Graubert, MD
Director, Cancer Center Program in Hematologic Malignancies
Hagler Family Endowed Chair in Hematologic Malignancies
Professor of Medicine

Wilhelm Haas, PhD
Assistant Professor of Medicine

Daniel A. Haber, MD, PhD

Nir Hacohen, PhD

Aaron Hata, MD, PhD
Assistant Professor of Medicine

Anthony John Iafrate, MD, PhD
Austin L. Vickery, Jr. Professor of Pathology
Deputy Chair, Department of Pathology

Othon Iliopoulos, MD
Associate Professor of Medicine

Max Jan, MD, PhD
Assistant Professor of Pathology

David M. Langenau, PhD
Atul K. Bhan, MBBS, MD, Endowed Chair in Experimental Pathology
Professor of Pathology (Molecular Pathology Unit)

Michael S. Lawrence, PhD
Assistant Professor of Pathology

Abner Louissaint, Jr., MD, PhD
Aziz and Nur Hamzaogullari Endowed Scholar in Hematologic Malignancies
Associate Professor of Pathology (Molecular Pathology Unit)

Shyamala Maheswaran, PhD
Mary B. Saltonstall Endowed Chair in Oncology
Professor of Surgery

Robert Manguso, PhD
Co-Director Tumor Immunotherapy Discovery Engine, Broad Institute
Assistant Professor of Medicine

Marcela V. Maus, MD, PhD
Director, Cancer Center Program in Cellular Immunotherapy
Paula J. O'Keeffe Endowed Chair in Thoracic Oncology
Associate Professor of Medicine

Andrea I. McClatchey, PhD

David T. Miyamoto, MD, PhD
Assistant Professor of Radiation Oncology

Mo Motamedi, PhD
James and Patricia Poitras Endowed Chair in Cancer Research
Assistant Professor of Medicine

Eugene Oh, PhD
Assistant Professor of Medicine

Christopher J. Ott, PhD
Assistant Professor of Medicine

Luca Pinello, PhD
Associate Professor in Pathology (Molecular Pathology Unit)

Esther Rheinbay, PhD
Assistant Professor of Medicine

Miguel N. Rivera, MD
Thomas F. and Diana L. Ryan MGH Research Scholar 2019-2024
Associate Professor of Pathology (Molecular Pathology Unit)

Debattama Sen, PhD
Assistant Professor of Medicine

Dennis C. Sgroi, MD
Executive Vice-Chair of Pathology
Professor of Pathology

Toshihiro Shioda, MD, PhD
Associate Professor of Medicine

Shannon Stott, PhD
d'Arbeloff MGH Research Scholar 2022-2027
Associate Professor of Medicine

Mario L. Suvà, MD, PhD
Vice-Chair of Pathology for Research
Director, Molecular Pathology Unit
Janet and William Ellery James MGH Research Scholar 2020-2025
Associate Professor of Pathology

David T. Ting, MD
Associate Clinical Director for Innovation, Cancer Center
Amin and Zebunisha Juma Endowed Chair in Oncology
Associate Professor of Medicine

Alexandra-Chloé Villani, PhD
Assistant Professor of Medicine (Center Immunology & Inflammatory Diseases)

Jackson Laboratories

Genevieve M. Boland, MD, PhD
Vice Chair for Research, Department of Surgery
MGH Research Scholar 2023-2028
Associate Professor of Surgery

Nir Hacohen, PhD

Russell Jenkins, MD, PhD
Assistant Professor of Medicine

Moshe Sade-Feldman, PhD
*Faculty Member**

Ioannis Sanidas, PhD
*Faculty Member**

Simches Laboratories

Nabeel Bardeesy, PhD
John R. Gallagher III and Katherine A. Gallagher Endowed Chair in Gastrointestinal Cancer Research
Associate Professor of Medicine

Priscilla Brastianos, MD
Terry and Jean de Gunzburg MGH Research Scholar 2021-2026
Associate Professor of Medicine (Neuro-Oncology)

Leif W. Ellisen, MD, PhD
Director, Cancer Center Program in Breast Medical Oncology
Nelson Family and Jerry Younger, MD Endowed Chair in Breast Cancer Research
Professor of Medicine

Konrad Hochedlinger, PhD
Gerald and Darlene Jordan Endowed Chair for the Center for Regenerative Medicine
Professor of Medicine (Genetics)

Hanno Hock, MD, PhD
Brant Carleton Endowed Chair in Acute Myeloid Leukemia Research
Assistant Professor of Medicine

William L. Hwang, MD, PhD
Assistant Professor of Radiation Oncology (Center for Systems Biology)

Peter Miller, MD, PhD
Assistant Professor of Medicine

Raul Mostoslavsky, MD, PhD

David A. Sweetser, MD, PhD
Chief of Medical Genetics and Metabolism, Department of Pediatrics
Leslie Meyer and Lewis Ball Holmes Chair in Genetics and Teratology
Assistant Professor of Pediatrics (Pediatrics, Genetics)

Shobha Vasudevan, PhD
Associate Professor of Medicine

*Assistant Professor appointment process intiated

Faculty Listing by Theme

Cancer Cell Biology

Liron Bar-Peled, PhD
Genevieve Boland, MD, PhD
Shawn Demehri, MD, PhD
Andrew Elia, MD, PhD
Konrad Hochedlinger, PhD
William L. Hwang, MD, PhD
David M. Langenau, PhD
Shyamala Maheswaran, PhD
Andrea I. McClatchey, PhD
Eugene Oh, PhD
Miguel Rivera, MD
Shobha Vasudevan, PhD

Cancer Genomics, Epigenetics and Proteomics

Liron Bar-Peled, PhD
Lloyd Bod, PhD
Genevieve Boland, MD, PhD
Priscilla Brastianos, MD
Andrew Elia, MD, PhD
Leif Ellisen, MD, PhD
Timothy Graubert, MD
Wilhelm Haas, PhD
Konrad Hochedlinger, PhD
Hanno Hock, MD, PhD
William L. Hwang, MD, PhD
Abner Louissaint, Jr., MD, PhD
Peter Miller, MD, PhD
David Miyamoto, MD, PhD
Raul Mostoslavsky, MD, PhD
Mo Motamedi, PhD
Eugene Oh, PhD
Christopher J. Ott, PhD
Luca Pinello, PhD
Esther Rheinbay, PhD
Miguel N. Rivera, MD
Debattama Sen, PhD
Mario L. Suvà, MD, PhD
David Sweetser, MD
David T. Ting, MD

Cancer Immunology

Lloyd Bod, PhD
Genevieve Boland, MD, PhD
Shawn Demehri, MD, PhD
David Fisher, MD, PhD
Francesca Gazzaniga, PhD
Nir Hacohen, PhD
Max Jan, MD, PhD
Russell Jenkins, MD, PhD
Robert Manguso, PhD
Marcela V. Maus, MD, PhD
Moshe Sade-Feldman, PhD
Debattama Sen, PhD
Alexandra-Chloé Villani, PhD

Cancer Metabolism

Liron Bar-Peled, PhD
Nabeel Bardeesy, PhD
Leif Ellisen, MD, PhD
Othon Iliopoulos, MD
Raul Mostoslavsky, MD, PhD

Genomic Instability

Andrew Elia, MD, PhD
Doğa Gülhan, PhD
Michael S. Lawrence, PhD
Peter Miller, MD, PhD
Shyamala Maheswaran, PhD
Raul Mostoslavsky, MD, PhD
Eugene Oh, PhD

Metastasis and Quiescence

Liron Bar-Peled, PhD
Nabeel Bardeesy, PhD
Priscilla Brastianos, MD, PhD
Daniel A. Haber, MD, PhD
Shyamala Maheswaran, PhD
Raul Mostoslavsky, MD, PhD
Mo Motamedi, PhD
Shobha Vasudevan, PhD

Molecular Cancer Diagnostics

Doğa Gülhan, PhD
Daniel A. Haber, MD, PhD
William L. Hwang, MD, PhD
A. John Iafrate, MD, PhD
David M. Langenau, PhD
Abner Louissaint, Jr., MD, PhD
Shyamala Maheswaran, PhD
David Miyamoto, MD, PhD
Miguel Rivera, MD
Dennis Sgroi, MD
Mario L. Suvà, MD, PhD
Shannon Stott, PhD
David T. Ting, MD

Molecular Therapeutics and Chemical Biology

Liron Bar-Peled, PhD
Ryan Corcoran, MD, PhD
Leif Ellisen, MD, PhD
Daniel A. Haber, MD, PhD
Aaron Hata, MD, PhD
A. John Iafrate, MD, PhD
David M. Langenau, MD, PhD
Christopher J. Ott, PhD
Ioannis Sanidas, PhD

Protein Degradation and Ubiquitin Signaling

Liron Bar-Peled, PhD
Andrew Elia, MD, PhD
William Hwang, MD, PhD
Max Jan, MD, PhD
Peter Miller, MD, PhD
Eugene Oh, PhD
Christopher J. Ott, PhD

RNA Biology

Mo Motamedi, PhD
Miguel N. Rivera, MD
David T. Ting, MD
Shobha Vasudevan, PhD

Systems and Computational Biology

Lloyd Bod, PhD
Gaddy Getz, PhD
Doğa Gülhan, PhD
Nir Hacohen, PhD
William L. Hwang, MD, PhD
Michael S. Lawrence, PhD
Mo Motamedi, PhD
Luca Pinello, PhD
Esther Rheinbay, PhD
Moshe Sade-Feldman, PhD
Toshihiro Shioda, MD, PhD

Brain Cancer
Priscilla Brastianos, MD
Andrew Elia, MD, PhD
A. John Iafrate, MD, PhD
Andrea I. McClatchey, PhD
Miguel N. Rivera, MD
Shannon Stott, PhD
Mario L. Suvà, MD, PhD

Breast Cancer
Liron Bar-Peled
Lloyd Bod, PhD
Shawn Demehri, MD, PhD
Andrew Elia, MD, PhD
Leif Ellisen, MD, PhD
Francesca Gazzaniga, PhD
Gaddy Getz, PhD
Doğa Gülhan, PhD
Daniel A. Haber, MD, PhD
William L. Hwang, MD, PhD
A. John Iafrate, MD, PhD
Shyamala Maheswaran, PhD
Mo Motamedi, PhD
Esther Rheinbay, PhD
Ioannis Sanidas, PhD
Dennis Sgroi, MD

Genitourinary Cancers
Daniel A. Haber, MD, PhD
Othon Iliopoulos, MD
Shyamala Maheswaran, PhD
David Miyamoto, MD, PhD
Mo Motamedi, PhD
Toshihiro Shioda, MD, PhD

Head and Neck Squamous Cell Cancer
Moshe Sade-Feldman, PhD

Hematologic Malignancies
Gad Getz, PhD
Timothy Graubert, MD
Hanno Hock, MD, PhD
Max Jan, MD, PhD
David M. Langenau, PhD
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Sarcoma
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Reports from the Principal Investigators

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Bar-Peled Laboratory

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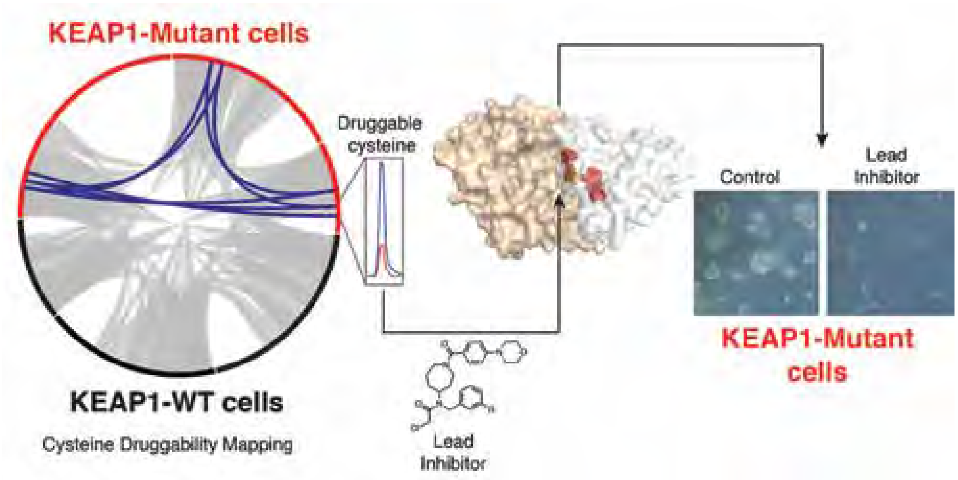
Research in **the Bar-Peled laboratory** sits at the interface of cellular metabolism and signal transduction and focuses on understanding how cancer cells respond to altered metabolic states. Rapidly proliferating cancer cells are characterized by increased production of toxic metabolic byproducts known 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 cancer 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 and may be exploited to develop chemical tools to inactivate these dependencies in cancers.

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 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



(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.

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:

Weiss-Sadan T, Ge M†, Hayashi M, Gohar M, Yao CH, de Groot A, Harry S, Carlin A, Fischer H, Shi L, Wei TY, Adelmann CH, Wolf K, Vornbäumen T, Dürr BR, Takahashi M, Richter M, Zhang J, Yang TY, Vijay V, Fisher DE, Hata AN, Haigis MC, Mostoslavsky R, Bardeesy N, Papagiannakopoulos T, **Bar-Peled L†**. NRF2 activation induces NADH-reductive stress, providing a metabolic vulnerability in lung cancer. *Cell Metab*. 2023 Apr 4;35(4):722.

Zhang J†, Simpson CM, Berner J, Chong HB, Fang J, Ordulu Z, Weiss-Sadan T, Possemato AP, Harry S, Takahashi M, Yang TY, Richter M, Patel H, Smith AE, Carlin AD, Hubertus de Groot AF, Wolf K, Shi L, Wei TY, Dürr BR, Chen NJ, Vornbäumen T, Wichmann NO, Mahamdeh MS, Pooladanda V, Matoba Y, Kumar S, Kim E, Bouberhan S, Oliva E, Rueda BR, Soberman RJ, Bardeesy N, Liau BB, Lawrence M, Stokes MP, Beausoleil SA, **Bar-Peled L†**. Systematic identification of anticancer drug targets reveals a nucleus-to-mitochondria ROS-sensing pathway. *Cell*. 2023 May 25;186(11):2361-2379

Chen AL, Lum KM, Lara-Gonzalez P, Ogasawara D, Cognetta AB 3rd, To A, Parsons WH, Simon GM, Desai A, Petrascheck M†, **Bar-Peled L†**, Cravatt BF†. (2019) Pharmacological convergence reveals a lipid pathway that regulates C. elegans lifespan. *Nature Chemical Biology*, 15(5), 453–462.

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.† (2019) Chemical Proteomics Identifies Druggable Vulnerabilities in a Genetically Defined Cancer. *Cell*, 171(3), 696–709.e23.

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.

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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 and patient-derived models to define the role of key gene mutations that drive these cancer types. Current projects focus on understanding the function of cancer genes in controlling the way cells modulate their growth and utilize energy in response to available nutrients. Additional studies are exploring how some therapies targeting key mutations initially cause tumor to stop growth and why resistance eventually develops. Each of these studies is being used to inform improved therapeutic approaches.

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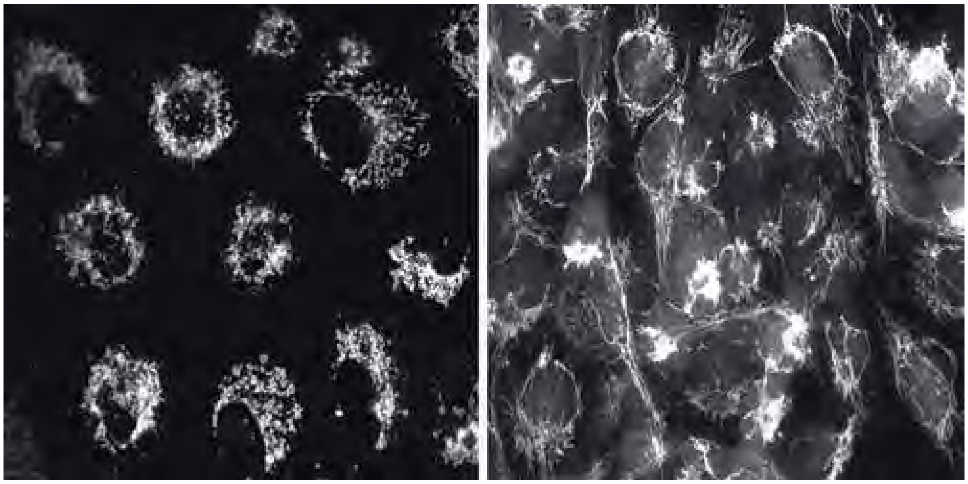
The Bardeesy lab studies the pathways driving the pathogenesis of pancreatic and biliary cancers. The lab has developed a series of genetically engineered mouse models that has elucidated the functional interactions of major gene mutations associated with these diseases in humans. Studies have focused on the roles of key cancer genes in regulation of cell metabolism, and the discovery of mechanisms of resistance to targeted therapies.

Interplay between metabolism and chromatin regulation

An important area of current focus in our lab is to elucidate the metabolic regulators of pancreatic cancer and biliary cancers, with particular attention paid to factors that reprogram cancer cell metabolism. We have linked mutations in the IDH1 gene to changes in metabolism that ultimately alter epigenetic states. Identifying these pathways has provided insights in mechanisms of cell transformation arising from these mutations and predict novel therapeutic vulnerabilities. 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 TET family DNA demethylases. We are focusing on how epigenetic defects caused by IDH-mediated inhibition of TET affect cross-talk between tumor and immune cells to support cancer growth.

Oncogenic functions of protein kinase A signaling in pancreatic and liver cancers

The protein kinase A (PKA) signaling pathway is activated by mutations in a number of tumor types. These include the subset of pancreatic and biliary tumors harboring mutations in GNAS, an upstream regulator of PKA, and a type of liver tumor (fibrolamellar carcinoma) harboring activating gene fusions of PKA. Although PKA is an important driver of the growth of these tumor types, the specific oncogenic mechanisms have not been as widely studied as for many other cancer gene mutations. We have focused on elucidating the primary mechanisms of PKA-driven growth. Our work has identified the Salt-inducible kinases (SIK1-3) as the critical targets of cancer-causing PKA alterations. In addition, we have linked this pathway to a downstream epigenetic mechanism controlling proliferation and reprogramming mitochondrial function and tumor cell metabolism.



Genetic control of expression of the Mitochondrial Fission Factor (MFF) dictates mitochondrial architecture and metabolic phenotypes of cancer cells. The image shows mitochondrial staining (Mitotracker) of cancer cells which express high levels of MFF (left panel) or low levels of MFF (right panel). The MFF-high cancer cells show hyper-fragmented mitochondria compared to the fused mitochondrial network of MFF-low cancers. This differential control of mitochondrial dynamics results in distinct metabolic programs and vulnerabilities.

Understanding and targeting FGFR2-driven biliary cancer

Genetic alterations that activate Fibroblast Growth Factor 2 (FGFR2) signaling are common in biliary cancer and predict response to pharmacological inhibition of the FGFR in patients. However, tumor shrinkage is often modest and acquired resistance invariably arises. We are investigating oncogenic mechanisms controlled by FGFR2 in biliary cancer, including direct targets of FGFR2 signaling as well as downstream impact on cellular metabolism and differentiation. Additionally, we are investigating resistance mechanisms and approaches to prevent and overcome resistance.

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 and the use of this system in large-scale genetic and small-molecule screens to systematically define targetable vulnerabilities in molecularly defined subtypes of this cancer.

Selected Publications:

Shi L, Shen W, Davis MI, Kong K, Vu P, Saha SK, Adil R, Kreuzer J, Egan R, Lee TD, Greninger P, Shrimp JH, Zhao W, Wei TY, Zhou M, Eccleston J, Sussman J, Manocha U, Weerasekara V, Kondo H, Vijay V, Wu MJ, Kearney SE, Ho J, McClanaghan J, Murchie E, Crowther GS, Patnaik S, Boxer MB, Shen M, Ting DT, Kim WY, Stanger BZ, Deshpande V, Ferrone CR, Benes CH, Haas W, Hall MD, **Bardeesy N**. SULT1A1-dependent sulfonation of alkylators is a lineage-dependent vulnerability of liver cancers. *Nat Cancer*. 2023 Mar;4(3):365-381.

Wu MJ, Shi L, Dubrot J, Merritt J, Vijay V, Wei TY, Kessler E, Olander KE, Adil R, Pankaj A, Tummala KS, Weerasekara V, Zhen Y, Wu Q, Luo M, Shen W, García-Beccaria M, Fernández-Vaquero M, Hudson C, Ronseaux S, Sun Y, Saad-Berreta R, Jenkins RW, Wang T, Heikenwälder M, Ferrone CR, Goyal L, Nicolay B, Deshpande V, Kohli RM, Zheng H, Manguso RT, **Bardeesy N**. Mutant IDH Inhibits IFNγ-TET2 Signaling to Promote Immunoevasion and Tumor Maintenance in Cholangiocarcinoma. *Cancer Discov*. 2022 Mar 1; 12(3):812-835.

Wu, Q, Zhen, Y, Shi, L. and **Bardeesy N**. EGFR inhibition potentiates FGFR inhibitor therapy and overcomes resistance in FGFR2 fusion-positive cholangiocarcinoma. *Cancer Discov.* 2022 Apr 14:OF1-OF18.

Cleary JM, Raghavan S, Wu Q, Li YY,...(**Bardeesy N***, Wolpin BM*). FGFR2 Extracellular Domain In-Frame Deletions are Therapeutically Targetable Genomic Alterations that Function as Oncogenic Drivers in Cholangiocarcinoma. *Cancer Discov*. 2021 Apr 29.

Goyal L, Shi L, Liu LY, Fece de la Cruz F,... Corcoran RB, **Bardeesy N**. TAS-120 Overcomes Resistance to ATP-Competitive FGFR Inhibitors in Patients with FGFR2 Fusion-Positive Intrahepatic Cholangiocarcinoma. *Cancer Discov*. 2019 Aug 9; (8):1064-1079.

Patra KC, Kato Y, Mizukami Y,... **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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Bod Laboratory

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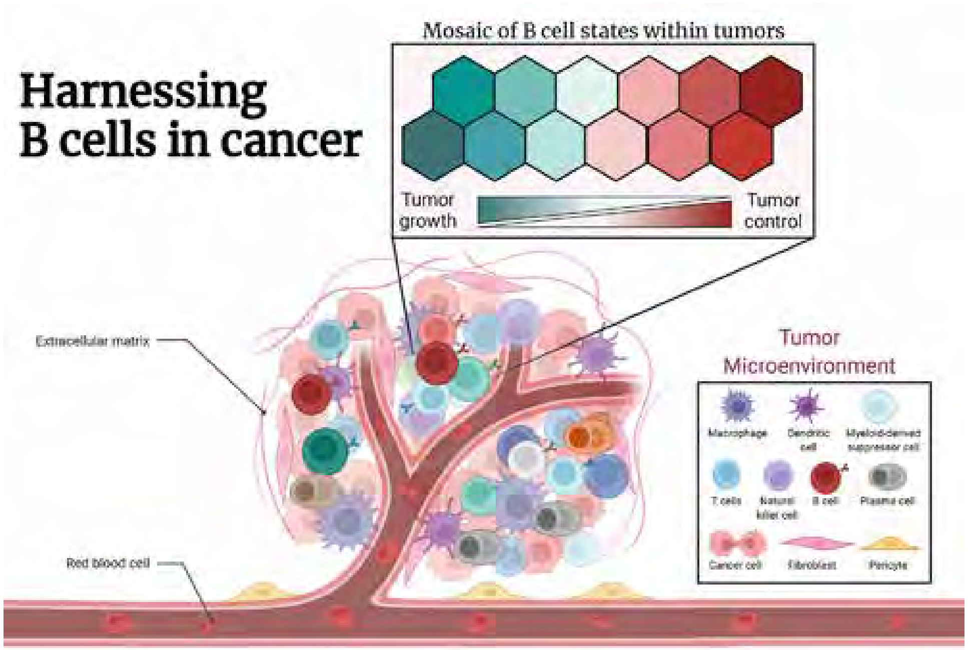
Immunotherapies have demonstrated remarkable clinical success in the treatment of various cancers mainly by boosting the function of endogenous T cells to attack neoplastic cells. Unfortunately, the frequency of patients responding to these therapies is modest and a significant fraction of patients develop severe immune-related adverse events. These observations have catalyzed a more thorough investigation of other cell types in the tumor microenvironment that could be targeted to increase treatment efficacy while mitigating toxicity. B cells are an important arm of the adaptive immune system frequently infiltrating solid tumors, however, their function on cancer progression has not been sufficiently explored. **The Bod laboratory** focuses on deciphering the landscape of phenotypic and functional B cell states within tumors. In particular, we are interested in exploring which B cell subset is favorable or detrimental for cancer progression, and by which mechanisms these B cells control tumor growth. Our thorough examination of the B cell response towards cancer aims to provide a new angle to harness the anti-tumor immune response more effectively.

Historically, B cells have been at the forefront of research in allergies, infectious diseases, and vaccines. Beyond mediating the humoral response, B cells are potent antigen presenting cells (APCs). They can provide co-stimulatory or co- inhibitory signals and secrete cytokines and chemokines that regulate functions of other cell types including effector T cells.

However, the role of B cells in the cancer scenario is unclear. While some studies have shown that B cells are critical for promoting anti-tumor immunity, others report that they may play a detrimental role, favoring relapse and metastasis. Indeed, on one hand, B cells form tertiary lymphoid structures (TLS) in the context of successful immune checkpoint blockade (ICB) therapy in human cancer patients, suggesting that B cells and TLS provide critical help to promote anti-tumor immunity and inhibit tumor growth.

On the other hand, B cells may also play an inhibitory role through the expression of soluble and/or inhibitory molecules on their surface which contribute to dismantle the anti-tumor T cell immunity. Whether the paradoxical effects of B cells in these settings is due to their functional diversity or distinct roles within different tumor types remains to be elucidated.

A more comprehensive understanding of B cell heterogeneity in tumors will allow us to identify B cell subsets and their respective functionality arising during different stages of tumor growth and regulating anti-tumor immunity. Growing evidence suggests that lymphocytes occupy a vast and continuous landscape of possible cellular states, as opposed to the idea of disconnected discrete subtypes. Recent advances in genomic analysis and sophisticated computational methods are enabling us to explore such diversity and are transforming our comprehension of immunology. Using such approaches, the lab aims to generate new insights into the role of B cells in inducing and regulating anti-tumor immunity. The main axes of research in our laboratory are:



While existing anti-cancer immunotherapies mainly engage effector T cells, harnessing both arms of the adaptive immune system might be more favorable. Illustrated by the mosaic of diverse B cell states, B cells are a highly dynamic cell population in the tumor microenvironment (TME) favoring or impeding tumor growth. In our lab, we want to thoroughly dissect the diverse and complex functions of TME-associated B cells to pave the way for new therapeutic avenues and improve the anti-cancer immune response. Adapted from "Tumor Microenvironment", by BioRender.com (2022).

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1. Deciphering the landscape of B cell states within the tumor microenvironment using multi-omics technologies. Our goal is to establish an atlas of B cell states in cancer, and to thoroughly interpret the spatial, transcriptomic, and epigenetic status of B cells in different contexts (e.g., different tumor types, healthy tissues, post-treatment with immune checkpoint blockade therapy, chemotherapy, or radiotherapy).
2. Identifying B cell-specific biomarkers and/or -targets in cancer. Using genetic and genomics approaches, we aim to explore potential B cell biomarkers and novel targets that are expressed on B cells, which may synergize with T cell-based checkpoint blockade therapy to enhance anti-tumor immunity.
3. Dissecting the underlying cellular and molecular mechanisms that govern the B cell response to cancer. The tumor microenvironment is layered with multiple tissular, cellular and molecular components which are associated with distinct tumor-promoting or -inhibiting mechanisms, and ultimately, open distinct therapeutic windows. We are interested in elucidating how B cells integrate these components and how the anti-tumor B cell response evolves in response to these signals.

Selected Publications:

Bod L, Kye YC, Shi J, Torlai Triglia E, Schnell A, Fessler J, Kuchroo JR, Barilla RM, Zaghouani S, Christian E, Delorey TM, Mohib K, Xiao S,Rothstein DM, Rozenblatt-Rosen O, Sharpe AH, Apetoh L, Regev A, Kuchroo V.K: B-cell specific checkpoint molecules that regulate anti-tumor immunity. In Press at *Nature* 2023.

Schnell A, **Bod L**, Madi A, Kuchroo VK. (2020). The yin and yang of co-inhibitory receptors: toward anti-tumor immunity without autoimmunity. *Cell Res*, 30(4), 285-299.

Xiao S, **Bod L**, Pochet N, Kota SB, Hu, D, Madi A, Kilpatrick J, Shi J, Ho A, Zhang H, Sobel R, Weiner HL, Strom TB, Quintana FJ, Joller N, Kuchroo VK. (2020). Checkpoint Receptor TIGIT Expressed on Tim-1(+) B Cells Regulates Tissue Inflammation. *Cell Rep*, 32(2), 107892.

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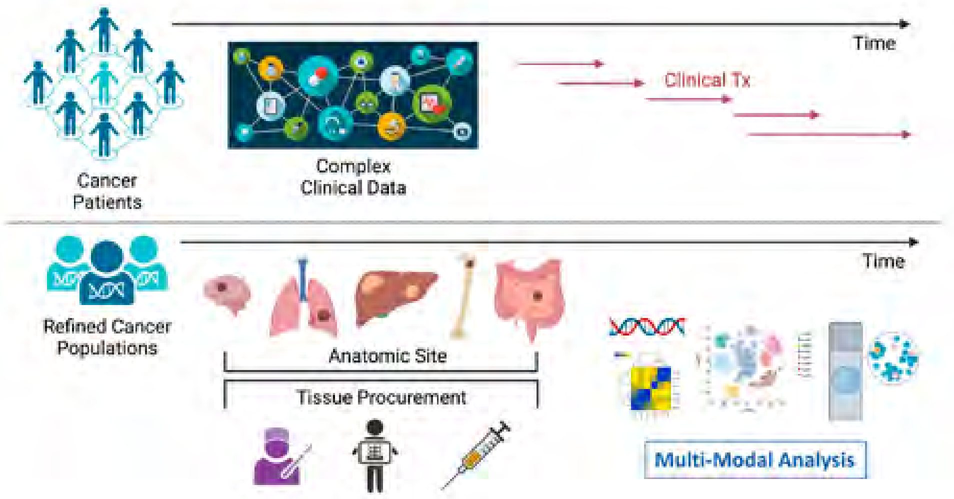
As a translational immuno-oncology laboratory, **the Boland laboratory** is focused on questions relating to tumor and immune interactions. The group uses a variety of complex approaches to characterize tumor biology and understand the interactions between tumor and immune cells and how these modify the surrounding tumor and tissue. Additionally, the Boland Lab is focused on identification of blood-based biomarkers to inform clinical decision-making. The areas of interest to the laboratory span from early cancer biology (why tumors form and/or metastasize) to how tumors respond to a variety of modern therapies. The Boland Lab bridges the complementary but disparate environments of clinical and basic research, with a primary goal of translating interesting research findings into meaningful clinical interventions based on the newest available technology.

The Boland Lab leads the Mass General correlative immuno-oncology efforts in melanoma and GI malignancies. The goal is to utilize patient-derived specimens (tumors/blood) to understand cancer biology, identify mechanisms of response and resistance to current therapies, identify biomarkers of therapeutic responses and immune-related toxicities, and nominate new targets for combinatorial trials. The group uses emerging technology to deconvolve tumor and immune interactions, integrating multiple complex datasets to understand the dynamic interplay occurring in the tumor microenvironment. The lab's translational research pipeline spans from clinical tissue and blood-based analyses to ex vivo tumor/immune modeling to small animal models of cancer. The focus of the Boland Lab is not limited to cutaneous melanoma but also includes rare melanoma subtypes and a variety of solid tumor histologies in which tumor-immune interactions are critical for tumor formation and propagation. Through these efforts, the Boland Lab has identified a de-differentiated tumor phenotype that is multi-drug resistant, and efforts are ongoing to target unique vulnerabilities in these cells thought to be responsible for downstream recurrences. Simultaneously, the Boland Lab has identified novel relationships between these resistant cell types and immune cells in the tumor microenvironment, allowing refinement of combinatorial therapeutic approaches.

In parallel, the Boland Lab is using the tumor-level analysis to identify and validate blood-based biomarkers allowing more cost-effective and clinically viable platforms to inform clinical decision making in real time. The approaches in the Boland Lab leverage extracellular vesicles, plasma proteomics, and immunophenotyping in parallel with integrated tumoral analysis for immunotherapy response prediction and monitoring, as well as for identifying and characterizing immune-related adverse events.

Finally, the group is focused on direct-to-tumor therapies, and Dr. Boland also serves as Director of the Therapeutic Intralesional Program. This component of the Boland Lab's efforts is directed toward clinical



The Boland Lab creates a translational pipeline arising directly from patient care and feeding back to next-generation clinical trials.

translation of research findings, with an emphasis on regionally applied therapies (e.g., oncolytic viruses, vaccines, ablative therapies). In this way, the Boland Lab is uniquely positioned between the clinical and translational realms, seamlessly creating a bidirectional pipeline informed by the clinical care of patients and feeding into the next generation of clinical trials.

Selected Publications:

Du K, Wei S, Wei Z, Frederick D, Mioa B, Moll T, Tian T, Sugarman E, Gabrilovich D, Sullivan R, Liu L, Flaherty K, **Boland GM***, Herlyn M*, Zhang G*. Pathway signatures derived from on-treatment tumor specimens predict response to anti-PD1 blockade in metastatic melanoma. *Nat Commun*. 2021 Oct 15;12(1):6023.

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Bai X, Kim M, Kasumova G, Si L, Tang B, Cui C, Yang X, Wei X, Cohen J, Lawrence D, Freedman C, Fadden R, Rubin K, Sharova T, Frederick D, Flaherty K*, Sullivan R*, Guo J*, **Boland G***. Radiological dynamics and SITC-defined resistance types of advanced melanoma during anti-PD-1 monotherapy: an independent single-blind observational study on an international cohort. *J Immunother Cancer*. 2021 Feb;9(2):e002092.

* Co-corresponding authors

Priscilla K. Brastianos, MD

The Brastianos laboratory studies molecular 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 the tumor and immune microenvironment 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 several 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.

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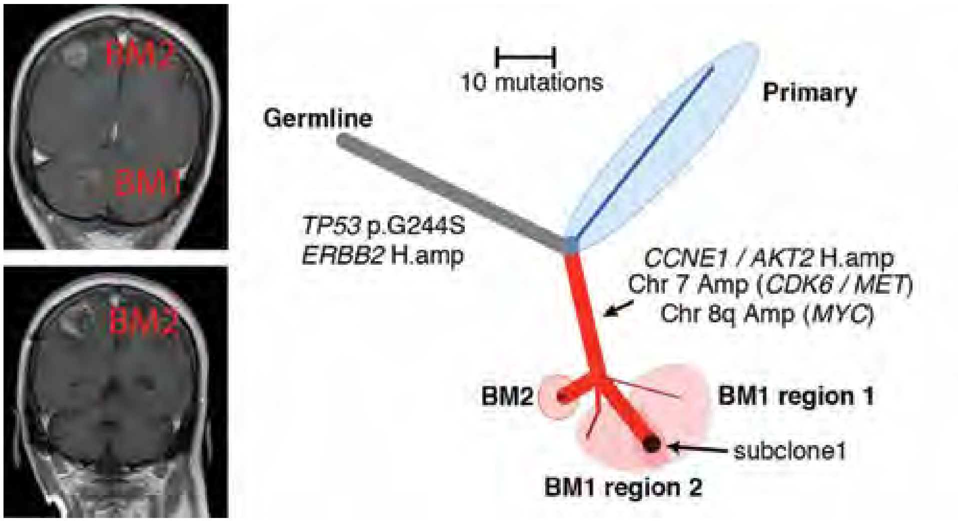
Characterizing genomic drivers of craniopharyngiomas

Craniopharyngiomas are a rare brain tumor that can cause profound clinical sequelae both through mass effect at presentation and through morbidity of treatment. Historically, 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 (Brastianos et al. *Nature Genetics* 2014). These findings have important implications for the diagnosis and treatment of these neoplasms. We initiated a national multicenter trial in craniopharyngiomas (Alliance A071601) to investigate the role of targeted therapies in these tumors. In patients with newly diagnosed papillary craniopharyngioma, we showed that all patients who received one or more cycles of vemurafenib/cobimetinib had dramatic responses to therapy (Brastianos et al. *NEJM* 2023)

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 and demonstrated that meningiomas harbor recurrent oncogenic clinically actionable mutations in AKT1 (E17K) and SMO (W535L) (Brastianos et al. *Nature Genetics* 2013). Notably, these mutations were present in therapeutically challenging tumors of the skull base. We also recently identified potential genetics drivers of progression in meningiomas (BAP1, TERT promoter mutations, DMD). Our lab is working on developing better preclinical models of meningioma with the goal of testing new therapeutic targets in this disease. We are now conducting a prospective national multicenter Phase 2 study (A071401) of targeted therapy in patients with recurrent or progressive meningiomas harboring clinically actionable mutations, respectively (Brastianos et al. *JCO* 2023).



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.

Central nervous system metastasis center

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 Center are to (1) identify novel therapeutic targets through comprehensive molecular 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. We are comprehensively characterizing the tumor and immune microenvironment of brain metastases to understand how they evolve in the CNS. We have demonstrated that brain metastases harbor clinically actionable drivers not detected in the primary tumors (Brastianos, Carter et al. *Cancer Discovery* 2015). We are evaluating the roles of these genetic alterations using various assays of metastasis (Shih, Nayyar et al. *Nature Genetics* 2020) and inhibiting pathways commonly altered in brain metastases with novel therapies. In addition, using single-cell RNA sequencing, we are characterizing the dynamic changes in immune microenvironment during treatment (Prakadan et al. *Nature Communications* 2021). Based on the work in the lab, we have now initiated a national genomically guided brain metastasis trial (A071701). 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:

Brastianos PK et al. BRAF-MEK Inhibition in Newly Diagnosed Papillary Craniopharyngiomas. *N Engl J Med*. 2023 Jul 13;389(2):118-126.

Alvarez-Breckenridge C, .. **Brastianos PK****, Carter SL.** Microenvironmental landscape of human melanoma brain metastases in response to immune checkpoint inhibition. *Cancer Immunol Res*. 2022 Jun 15:canimm.CIR-21-0870-E.2021.

Prakadan SM, Alvarez-Breckenridge C.A., Markson SC, … Carter SL**, **Brastianos PK****, Shalek AK**. Genomic and transcriptomic correlates of immunotherapy response within the tumor microenvironment of leptomeningeal metastases. *Nat Commun*. 2021 Oct 12;12(1):5955

Brastianos PK*, Kim AE*, et al. Palbociclib demonstrates intracranial activity in progressive brain metastases harboring cyclin-dependent kinase pathway alterations. *Nature Cancer*. 2021; May;2 (5):498-502.

Shih DJH, Nayyar N,…Carter SL*, **Brastianos PK***. Genomic characterization of human brain metastases identifies drivers of metastatic lung adenocarcinoma. *Nat Genet*. 2020; Apr;52(4):371-377.

Brastianos PK, et al. Single-arm, open-label phase 2 trial of pembrolizumab in patients with leptomeningeal carcinomatosis. *Nat Med*. 2020; Aug;26(8):1280-1284.

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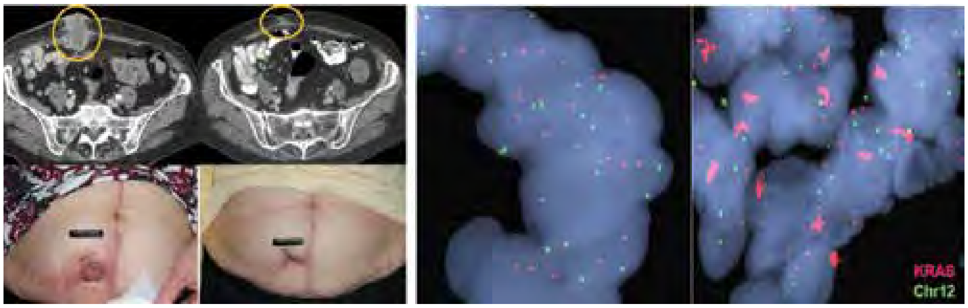
The Corcoran laboratory focuses on developing new and effective therapies for gastrointestinal cancers, including colorectal, pancreatic, stomach, and esophageal cancers, by targeting the specific survival signals that are active in a given patient's cancer. Our research utilizes targeted therapies, 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.

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 because KRAS itself has proven difficult to target directly with small molecules. Currently, our 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. We have identified adaptive feedback signals that impede the ability of MEK inhibitors to suppress MAPK signaling and have explored the role of novel agents (ERK inhibitors) or convergent signaling nodes to overcome feedback. We have expanded these approaches to identify other potentially effective targets in KRAS-mutant cancers, including direct KRAS inhibitors. Despite promising clinical responses in KRAS-G12C mutant NSCLC, there has been limited efficacy of G12C inhibitors as single agents in colon cancer. To address this limitation, we have defined key mechanisms of adaptive feedback resistance in response to KRAS inhibition and have employed vertical pathway inhibition strategies targeting the RAS-MAPK pathway as described in a recent publication (*Clinical Cancer Research*, 2020).

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:

Tian J, Chen JH, Chao SX, Pelka K, Giannakis M, Hess J, Burke K, Jorgji V, Sindurakar P, ..., Demehri S, Leary R, Campbell CD, Yilmaz O, Getz GA, Parikh AR, Hacohen N, **Corcoran RB**. Combined PD-1, BRAF and MEK inhibition in $BRAF^{V600E}$ colorectal cancer: a phase 2 trial. *Nat Med*. 2023 Feb;29(2):458-466.

Ryan MB, Coker O, Sorokin A, Fella K, Barnes H, Wong E, Kanikarla P, Gao F, Zhang Y, Zhou L, Kopetz S, **Corcoran RB**. KRASG12C-independent feedback activation of wild-type RAS constrains KRASG12C inhibitor efficacy. *Cell Rep*. 2022 Jun 21;39(12):110993. doi: 10.1016/j.celrep.2022.110993.

Tanaka N, Lin JJ, Li C, Ryan MB, Zhang J, Kiedrowski LA, Michel AG, Syed MU, Fella KA, Sakhi M, Baiev I, Juric D, Gainor JF, Klempner SJ, Lennerz JK, Siravegna G, Bar-Peled L, Hata AN, Heist RS, **Corcoran RB**. Clinical acquired resistance to KRASG12C inhibition through a novel KRAS switch-II pocket mutation and polyclonal alterations converging on RAS-MAPK reactivation. *Cancer Discov.* 2021 Aug;11 (8):1913-1922.

Ryan MB, Fece de la Cruz F, Phat S, Myers DT, Wong E, Shahzade HA, Hong CB, **Corcoran RB**. Vertical pathway inhibition overcomes adaptive feedback resistance to KRASG12C inhibition. *Clin Cancer Res*. 2020. 26:1633-1643.

Parikh AR*, Leshchiner I*, Elagina L*, Goyal L, Levovitz C, Siravegna G,... Iafrate AJ, Adalsteinsson VA, Bardelli A, Parida L, Juric D, Getz G, **Corcoran RB**. Liquid versus tissue biopsy for detecting acquired resistance and tumor heterogeneity in gastrointestinal cancers. *Nature Medicine*/2019 Sep;25(9):1415-1421).

Corcoran RB, André T,...Rangwala F, Van Cutsem E. Combined BRAF, EGFR, and MEK Inhibition in Patients with BRAFV600E-Mutant Colorectal Cancer. *Cancer Discovery.* 2018; 8: 428-443.

*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 the Demehri 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 prevention and therapy.

Demehri Laboratory

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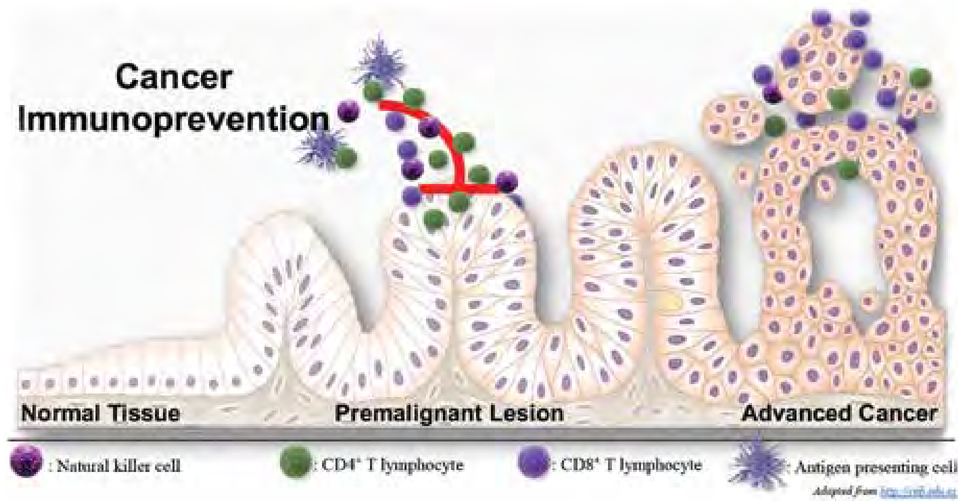
The field of cancer immunology has made substantial advances in recent years by deciphering the role of the tumor infiltrating CD8+ cytotoxic T lymphocytes (CTLs) in attacking cancer cells, which have led to promising new cancer immunotherapeutics. The current immunotherapeutic approaches, however, are 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 studies the role of alarmins, damage-associated molecular patterns (DAMPs)/stress signals, commensal viruses, carcinogens, and aging-associated factors in regulating early cancer development. The major areas of research in our laboratory are:

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 cancer development in vivo remains unclear. Our laboratory utilizes an NK cell-specific activating ligand 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



Immune Regulation of Early Cancer Development.

by the activated NK cells to block cancer promotion and progression.

3) The impact of commensal viruses-immune system interplays on the homeostasis of the organs exposed to environmental carcinogens. We aim to determine how the immune system's control of commensal virome regulates the homeostasis of the virus-colonized tissues. Through this effort, we aim to realize the beneficial functions of commensal virome for the prevention and treatment of cancer and other chronic diseases that affect humans.

4) Mechanisms of cancer promotion by the immune system. Although immune cells can mount anti-tumor immunity against cancer, they are also implicated in promoting cancer development in chronic inflammation. Our laboratory studies the initiating mechanisms of cancer-prone chronic inflammation development in the skin, pancreas, colon and liver, which are the major organs affected by chronic inflammation and its cancer sequela.

Selected Publications:

Hasegawa T, Oka T, Son HG, Oliver-García VS, Azin M, Eisenhaure TM, Lieb DJ, Hacohen N, **Demehri S**. Cytotoxic CD4+ T cells eliminate senescent cells by targeting cytomegalovirus antigen. *Cell*. 2023 Mar 30;186(7):1417-1431.e20.

Boieri M, Malishkevich A, Guennoun R, Marchese E, Kroon S, Trerice KE. Awad M, Park J H, Iyer S, Kreuzer J, Haas W, Rivera MN, **Demehri S**. CD4+ T helper 2 cells suppress breast cancer by inducing terminal differentiation. *J Exp Med*. 2022 Jul 4;219(7)

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Li K, Li T, Feng Z, Huang M, Wei L, Yan Z, Long M, Hu Q, Wang J, Liu S, Sgroi DC, **Demehri S**. CD8(+) T cell immunity blocks the metastasis of carcinogen-exposed breast cancer. *Science Advances*. 2021 June 18; 7(25): eabdB936.

Strickley JD, Messerschmidt JL, Awad ME, Li T, Hasegawa T, Ha DT, Nabeta HW, Bevins PA, Ngo KH, Asgari MM, Nazarian RM, Neel VA, Jenson AB, Joh J, and **Demehri S**. Immunity to commensal papillomaviruses protects against skin cancer. *Nature*. 2019 Nov;575(7783):519-522.

Cunningham TJ, Tabacchi M, Eliane JP, Tuchayi SM, Manivasagam S, Mirzaalian H, Turkoz A, Kopan R, Schaffer A, Saavedra AP, Wallendorf M, Cornelius LA, and **Demehri S**. Randomized trial of calcipotriol combined with 5-fluorouracil for skin cancer precursor immunotherapy. *J Clin Invest* 2017; 127(1): 106-116.

Andrew Elia, MD, PhD

Elia Laboratory

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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, focusing on pathways regulated by ubiquitin-dependent signaling and 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.

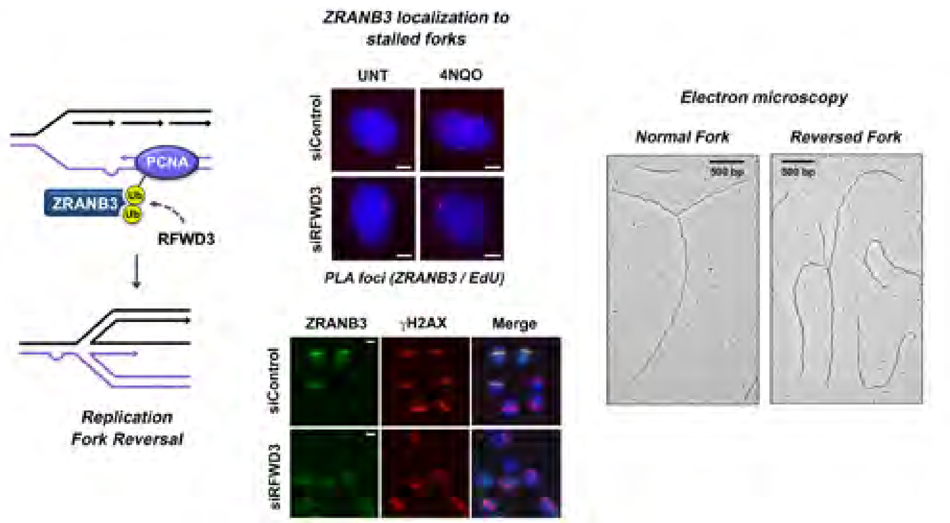
Ubiquitin signaling in the DNA damage response

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 can lead to hereditary cancer syndromes and can underlie the genomic instability which is a hallmark of 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. Execution of the DDR relies upon a dynamic array of protein modifications, with ubiquitination playing a central role. Our lab elucidates ubiquitin-dependent signaling pathways that regulate and integrate diverse DDR factors.

Replication-coupled repair 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 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 factor RPA to promote homologous recombination at stalled replication forks and replication fork restart (*Mol Cell* 2015b).

Replication fork reversal is an important mechanism to protect the stability of stalled forks. While multiple enzymes have been identified that can remodel forks, their regulation remains poorly understood. We have recently discovered a new function for RFWD3 in the regulation of fork remodeling (*J Cell Biol* 2023). We have found that RFWD3 promotes PCNA polyubiquitination to recruit the DNA translocase ZRANB3 to stalled replication forks. Through the analysis of replication intermediates by electron microscopy, we found that RFWD3 promotes replication fork reversal in a ZRANB3-epistatic manner. We are continuing to elucidate novel mechanisms of replication-coupled repair and fork stabilization regulated by ubiquitin signaling.



RFWD3 promotes PCNA polyubiquitination to recruit the DNA translocase ZRANB3 to remodel stalled replication forks (J Cell Biol 2023).

Quantitative proteomics

Numerous ubiquitin ligases have been implicated in the DNA damage 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.

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 treatments and immunotherapy. 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 and immunotherapy agents. We are employing methods to translate our work to the development of such therapies.

Selected Publications:

Moore CE, Yalcindag SE, Czeladko H, Ravindranathan R, Wijesekara Hanthi Y, Levy JC, Sannino V, Schindler D, Ciccia A, Costanzo V, **Elia AE**. RFWD3 Promotes ZRANB3 Recruitment to Regulate the Remodeling of Stalled Replication Forks. *J Cell Biol*, 2023; 222(5): e202106022

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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.

Elia AE, Cantley LC, Yaffe MB. Proteomic screen finds pSer/pThr-binding domain localizing Plk1 to mitotic substrates. *Science*. 2003; 299:1228-31.

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.

Leif William Ellisen, MD, PhD

Our laboratory specializes in work at the interface between basic tumor biology and therapeutic application. Understanding how key genes and pathways trigger the early, stepwise progression of cancer will be essential to moving beyond incremental steps and toward revolutionary advances in cancer treatment and prevention. **The Ellisen laboratory** is broadly interested in identifying such 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 prevention and therapeutic strategies for breast and other cancers.

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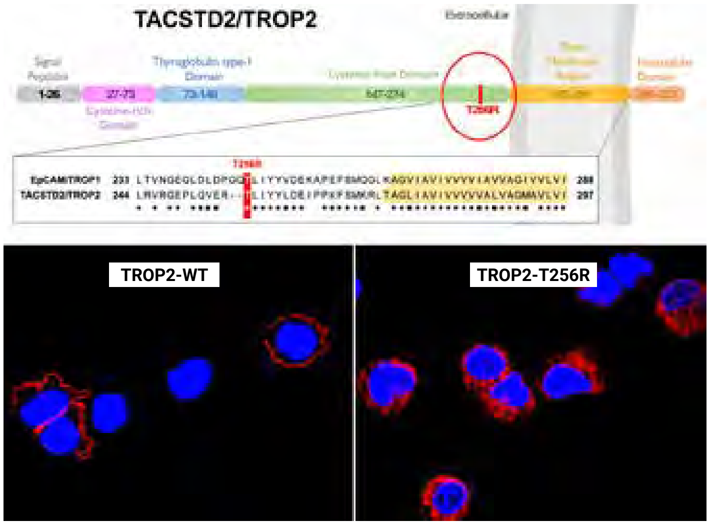
Our laboratory has a broad interest in how genetic abnormalities in breast cancer and related malignancies influence tumor biology, and how that biology can, in turn, be exploited to therapeutic advantage. We address these questions through basic research studies of key cancer drivers including DNA repair defects through BRCA1/2 and related pathways, and transcriptional reprogramming through the p53 gene family. Supporting and complementing these studies are sophisticated analyses of patient-derived precancerous and cancerous tissues. Recent innovative tissue-based studies have led to our discovery of novel cancer drivers, and have provided a unique window on early cancer pathogenesis, intratumoral heterogeneity and therapeutic resistance. 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. For more details please see our website, Ellisenlab.com.

Novel drivers of aggressive breast cancer subtypes

Our work employing advanced tumor molecular diagnostics has revealed gene fusions as novel drivers of an aggressive breast cancer subset. In triple-negative breast cancer (TNBC), extensive intratumoral heterogeneity is itself a driver that we have characterized through single-cell genomic and transcriptomic analysis, leading to our discovery of unanticipated drug resistance mechanisms with immediate therapeutic implications. Of particular interest is resistance to novel Antibody Drug Conjugates that are transforming cancer therapy. Our longstanding work on the biology of TNBC is supported by the institution- wide Triple-Negative Breast Cancer Program, which 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.



TROP2 is a cell-surface protein selectively expressed on tumor cells and targeted by emerging therapeutics including the antibody-drug conjugate sacituzumab govitecan (SG). Immunofluorescence (bottom) for TROP2 (red) in TNBC cells shows that the novel resistance mutation T256R results in TROP2 cytoplasmic mislocalization, which prevents SG binding.

BRCA1/2, hereditary cancer predisposition and prevention

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. Together with an international team of collaborators we are carrying out systematic studies 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. For example, our published single-cell genome analysis has revealed extensive chromosomal damage in BRCA-mutant breast tissues that precedes any histological abnormalities. This seminal finding implies the existence of early cellular defects and associated vulnerabilities that could be exploited for cancer prevention in this setting.

The p53 family network in cancer biology and therapy

The p53 tumor suppressor is inactivated in more than 50% of sporadic human cancers, and heterozygous germline p53 mutation confers striking tumor predisposition. As a transcription factor and key nodal point for integrating cellular stress responses, p53 controls diverse cellular processes including cell cycle progression, survival and metabolism. Through analysis of two p53-related genes, p63 and p73, we and others have defined a functional network and have further defined a tissue-specific role for p63 as the enforcer of an epigenetically-controlled stem/progenitor state. Tumor-selective deregulation of p63 and associated chromatin remodeling factors reprograms the transcriptome to inhibit differentiation, and promote immune evasion. These findings likely explain the observation that p63 is over-expressed in a large variety of epithelial tumors, particularly squamous cell and breast carcinomas. Collectively, this work serves as a paradigm for analysis of transcriptional reprogramming in cancer.

Selected Publications:

Coates JT, Sun S, Leshchiner I, Thimmiah N, Martin EE, McLoughlin D, Danysh BP, Slowik K, Jacobs RA, Rhrissorrakrai K, Utro F, Levovitz C, Denault E, Walmsley CS, Kambadakone A, Stone JR, Isakoff SJ, Parida L, Juric D, Getz G, Bardia A, and **Ellisen LW**. Parallel genomic alterations of antigen and payload targets mediate polyclonal acquired clinical resistance to sacituzumab govitecan in triple-negative breast cancer. *Cancer Discovery.* 2021 11:1-10.

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Fisher Laboratory

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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.

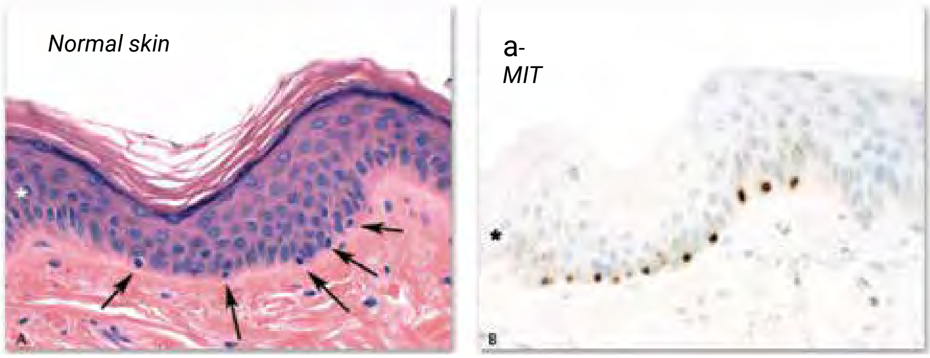
Our group studies cell death/proliferation signals in relation to development and disease, particularly in cancer of pigment cells (melanoma) and tumors of childhood. We attempt to understand critical modes of cell homeostasis with a goal of molecular targeted therapy as well as prevention of melanoma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



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.

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:

Choi YS, Erlich TH, von Franque M, … Demehri S, Hawryluk EB, and **Fisher DE**. Topical therapy for regression and melanoma prevention of congenital giant nevi. *Cell* 2022 Jun 9 185(12): 2071-2085

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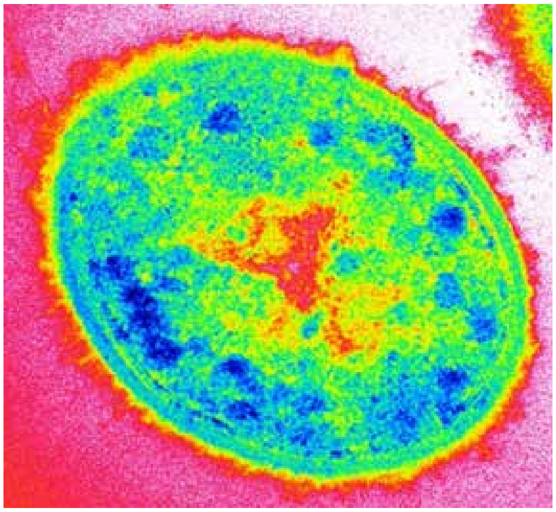
Gut microbiota — the trillions of bacteria, fungi, viruses, and archaea that reside in our gut — contain a dynamic arsenal of products that can protect from or contribute to disease. Diet, medication, exercise and disease impact the composition of the microbiota and influence the products the microbes produce. In turn, specific microbes influence immune cell function in both normal and disease states. **The Gazzaniga laboratory** focuses on unraveling this complex ecosystem that holds huge therapeutic potential, and that reveals the dynamic interplay of environmental factors, microbes, microbial products and immune cells. Specifically, we focus on three main questions: (1) Which bacteria are associated with response in cancer patients? (2) Which gut bacterial produced molecules impact anti-tumor immunity? (3) How do microbe-mediated immune responses impact the anti-tumor response to immunotherapy? Our ultimate goal is to uncover mechanistic information to develop microbe-based therapies that fine-tune the immune system to fight cancer.

The trillions of bacteria that inhabit our intestinal tract as part of our gut microbiota have a dynamic relationship with our immune system. For example, the bacteria in the gut impact the anti-tumor response of immune checkpoint inhibitors on tumors outside of the gut. Treatment with checkpoint inhibitors, such as antibodies targeting programmed cell death protein 1 (PD-1) or programmed cell death ligand 1 (PD-L1), disrupts interactions between PD-1 on T cells and PD-L1 on tumors, reinvigorating T cells to kill cancer cells. Although checkpoint inhibitors are used to treat a wide variety of cancers, the response rates are variable. Understanding what impacts the efficacy of checkpoint inhibitors is critical to increase the number of patients who respond to treatment.

Fecal transplants from melanoma patients who responded to PD-1 blockade can overcome resistance in non-responders. However, the efficacy of the fecal transplants varies with different donors, highlighting the need to understand how bacteria impact anti-tumor immunity. The purpose of the Gazzaniga lab is to translate the notion that the microbiome plays a role in anti-tumor immunity into reliable, microbiome-inspired treatments that increase the number of patients who respond to checkpoint blockade.

Patient stool samples: What is associated with response?

Many studies examining the role of the gut microbiome in response to checkpoint blockade therapy focused on melanoma. However, PD-1 blockade is approved for over 25 different cancers. Depending on the cancer type, PD-1 blockade efficacy ranges from 2%-87%. Therefore, understanding how the microbiome impacts the anti-tumor responses of checkpoint blockade in other cancers is critical to increase the number of patients who respond. We collaborate with clinicians at MGB to analyze stool samples from patients with different cancers at the beginning and end of treatment with checkpoint inhibitors. We investigate which treatments impact the gut microbiome and which bacteria are associated with anti-tumor responses in different cancers.



We isolated Erysipelatoclostridium ramosum from healthy human microbiota and found that it promotes an anti-tumor response to anti-PD-L1 therapy. We are currently isolating the anti-tumor molecule it produces and are investigating the immune pathways it impacts to promote anti-tumor immunity.

Searching for patient-derived therapeutics: What bacterial molecules promote anti-tumor immunity?

Many have sought to identify individual bacteria that could be used as probiotics in the clinic to promote anti-tumor immunity. However, several obstacles make probiotics an unreliable therapy. There are difficulties in delivering live anaerobic bacteria, difficulties in engraftment of probiotics in humans already colonized with bacteria, and differences between lab culture conditions and the human intestine that could contribute to the anti-tumor activity of the bacteria. Bacterial molecules, on the other hand, can be delivered and tested more reproducibly and thus bypass the variability of probiotics and fecal transplants. Using germ-free mice, which lack all microbes, we can investigate how different bacteria impact tumor outcomes. We have isolated two bacterial strains from a healthy human microbiome that promote anti-tumor immunity to PD-1 blockade and are currently identifying the anti-tumor molecules they produce. Next, we will isolate bacterial molecules from patient responder stool to develop reproducibly delivered patient-derived bacterial therapeutics to increase the efficacy of checkpoint inhibitor therapy.

Learning from bacteria: Which microbe-mediated immune mechanisms can we harness to promote anti-tumor immunity?

By comparing mice colonized with healthy human microbiota to mice treated with broad spectrum antibiotics, we have identified several immune pathways in the tumor-draining lymph nodes that are impacted by gut bacteria and associated with anti-tumor immunity. By targeting these immune pathways, we can convert non-responders to responders in multiple tumor models. To make our mouse models more clinically relevant, we compare mice colonized with patient non-responder or responder microbiota to identify immune pathways impacted only by responder microbes. Our overall goal is to learn from bacteria and develop therapeutics that target the immune pathways impacted by responder microbiota to increase the number of patients who respond to treatment.

Selected Publications:

Park JS*, **Gazzaniga FS***, Wu M, Gillis J, Zheng W, LaFleur MW, Johnson SB, Morad G, Park EM, Zhou Y, Watowich SS, Wargo JA, Freeman GJ**, Kasper DL**, Sharpe AH**. Targeting PD-L2–RGMb overcomes microbiome-related immunotherapy resistance. *Nature*. 2023 Jun 28.

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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 cancer (starting from normal cells and progressing to premalignancy, primary cancer, and emergence of resistance), comparing events at the DNA, RNA, and protein levels between one or more tumor and normal 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 drive cancer progression, drive resistance, or increase its risk as well as identifying molecular subtypes of the disease, their markers, and relationship to clinical variables. Recently, the Getz lab is also studying the tumor and its immune microenvironment using both bulk and single-cell RNA-sequencing (RNA-seq) data. In addition to developing tools for high throughput analysis of cancer data and experimentally testing the findings, the Getz lab develops computer platforms that enable large-scale analytics and visualization.

Characterizing the cancer genome

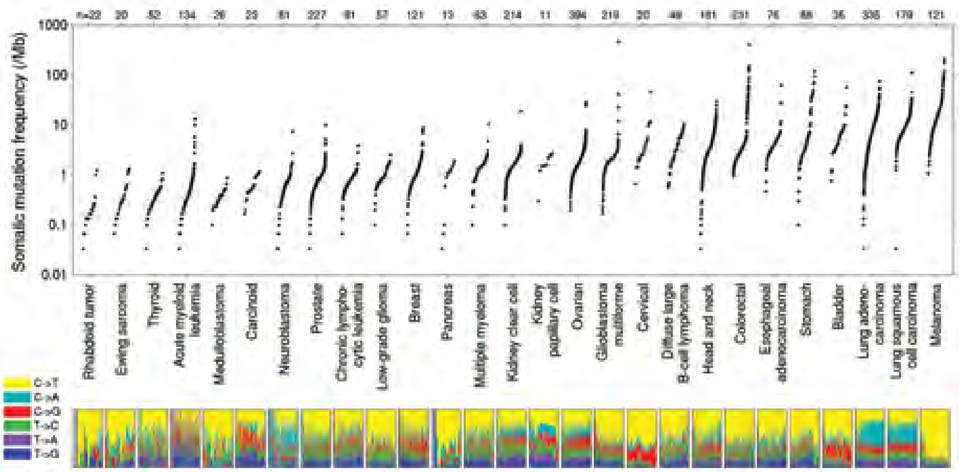
Cancer is a disease of the genome driven by a combination of possible germline risk-alleles, together with a few 'driver' somatic mutations that increase fitness and promote clonal expansion. Mutations occur at all levels and scales, including (i) DNA point mutations; (ii) small insertions and deletions;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

Detecting cancer-associated genes

After detecting genomic events, we search for genes (and pathways) that show significant signals of positive selection (e.g., the number of mutations exceeds what is expected by chance) across a cohort of samples by constructing a detailed statistical model of the background mutational processes and detecting genes that deviate from it. We developed tools to discover genes significantly gained or lost (GISTIC), and genes with increased density or irregular mutational patterns (MutSig, CLUMPS). In these analyses, correctly modeling the heterogeneity of mutational processes across patients, sequence contexts, and the genome is critical. We are constantly improving methods and working towards a unified method for all types of alterations. We also discovered drivers in non-coding regions of the genome in breast cancer (e.g., hotspot mutations in FOXA1 promoter that likely alter its expression) and, more recently, across cancer, as part of a large international effort.

Heterogeneity and clonal evolution of cancer

Cancer samples are heterogeneous: non-cancer cells intermingle with a cancer cell population that typically contains multiple subclones. Since cancer is a dynamic



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).

system, these subclones may represent (i) remaining cells of less-fit clones not yet overtaken by the expanding the most-fit clone, (ii) interacting subclones that co-evolved and have reached an equilibrium, or (iii) a combination of both. We have developed tools (ABSOLUTE, PhylogicNDT) to characterize the heterogeneity and dynamics of cancer using copy-number, mutational, and other data measured on bulk samples and single cells. These tools can analyze multiple samples per patient to infer clonality of mutations, number of subclones, and subclonal evolution over time or space. We previously demonstrated that subclonal driver mutations are associated with outcome, emphasizing the importance of including clonal information in clinical trials. By analyzing RNA-seq, we recently showed that most healthy adult tissues contain genetic clones with somatic mutations, some in known cancer-associated genes.

Mutational processes

Processes that damage, repair, replicate, and deliberately alter DNA create mutations. Mutation data can thus be used to study these processes, understand their mutational “signatures,” infer their molecular mechanisms, and identify alterations associated with their activity. By studying asymmetries in mutational processes, we detected a mechanism that acts on the lagging DNA strand during replication and a new mutational process that generates mutations on the non-transcribed strand. We also used the association between a mutational signature and homologous recombination (HR) defects to show that epigenetic silencing of RAD51C within the HR pathway is an important mechanism for HR deficiency in breast cancer. With international collaborators, we are mapping all common mutational signatures affecting single- and di-nucleotide substitutions as well as small insertions and deletions (indels). We also study indels that occur at microsatellites and, in particular, tumors that have microsatellite instability (MSI) that may benefit from immune checkpoint inhibitor treatment (e.g., anti-PD1). We are developing a method to computationally detect the presence of MSI tumors from cell-free DNA (cfDNA) containing DNA shed from tumor cells, easily obtained from non-invasive blood biopsies.

Selected Publications:

Leshchiner I, Mroz EA, Cha J, ..., **Getz G**, Rocco JW. Inferring early genetic progression in cancers with unobtainable premalignant disease. *Nat Cancer.* 2023 Apr;4(4):550-563.

Knisbacher BA, Lin Z, Hahn CK, Nadeu F, Duran-Ferrer M, ..., Martín-Subero JI, Puente XS, Stilgenbauer S, Wu CJ, Campo E, **Getz G**. Molecular map of chronic lymphocytic leukemia and its impact on outcome. *Nat Genet*. 2022 Nov;54(11):1664-1674.

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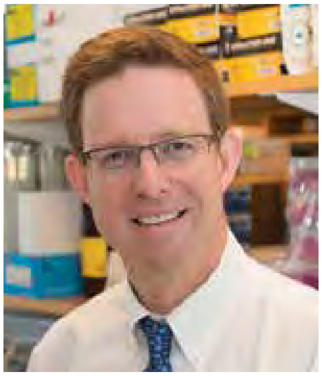

The Graubert laboratory focuses on the molecular basis of human blood cancers, including acute myeloid leukemia and myelodysplastic syndromes. The laboratory utilizes a variety of genomic platforms to interrogate primary samples from patients with myeloid malignancies to identify inherited and somatic 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

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Clonal heterogeneity of myelodysplastic syndromes

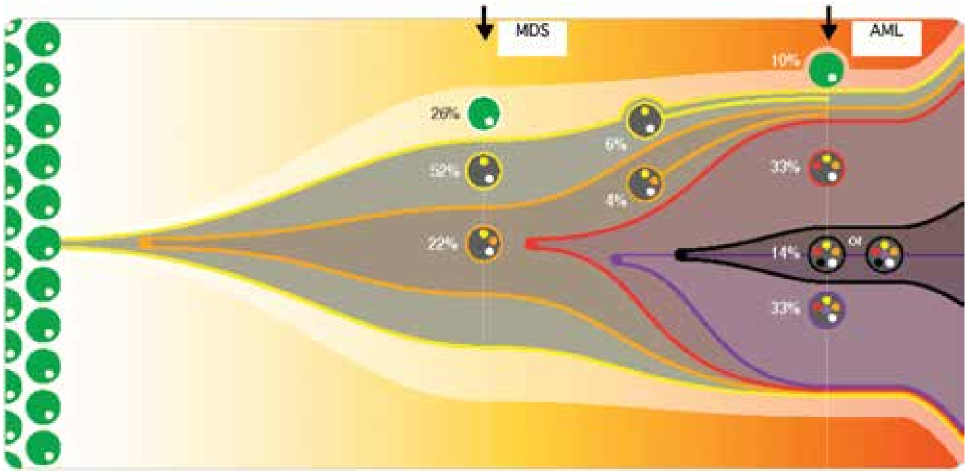
Myelodysplastic syndromes are the most common form of acquired bone marrow failure in adults. Despite the ineffective hematopoiesis that is characteristic of this disease in its early stages, we found through whole genome sequencing that nearly all cells in the bone marrow of these patients are clonally 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, 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



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.

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, Zou L, and **Graubert TA**. Targeting R-loop Associated ATR Response in Myelodysplastic Syndrome. *Oncotarget.* 2019 Apr 5; 10(27):2581-2582.

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Doğa C. Gülhan, PhD

Gülhan Laboratory
(Opens fall 2023)

Doğa C. Gülhan, PhD

In **the Gulhan laboratory**, we develop computational methods to advance personalized oncology by employing statistical and machine learning models to dissect the complexity of cancer genomes. Leveraging signature analysis techniques, we detect mutational patterns representative of genomic instability mechanisms, such as dysfunctional DNA repair or cell-cycle checkpoint pathways, based on which we produce a refined map of their subtypes. In collaboration with clinical researchers, we study the differences in targeted therapy outcomes for tumors displaying these mechanisms and their specific evolutionary trajectories leading to resistance. Genomic instability may also trigger anti-tumor immune responses or promote immune evasion. We analyze these connections to maximize the efficacy of treatments, in particular that of immunotherapies. Our goal is to develop computational methods that can achieve a more accurate interpretation of cancer genomes and use these advancements to tailor tools with clinical applications.

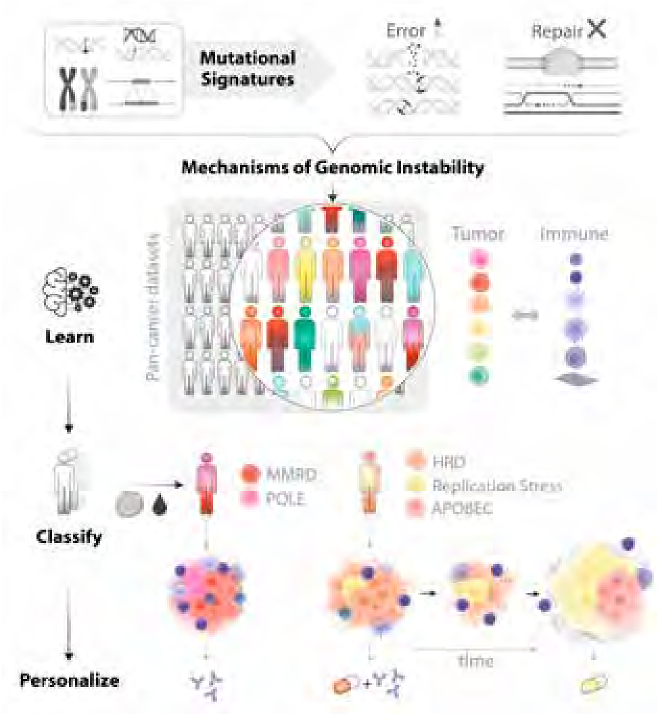
While cancer genomics offers deep insights into tumor landscapes, its full clinical potential remains untapped. Currently, personalized treatments cater to only a fraction of patients. Expanding the clinical interpretation of cancer genomes is essential to bridge this gap.

Genomic instability to guide treatments

Cancer cells have elevated mutation rates arising from a blend of factors like exogenous mutagens and intrinsic genomic instability. The latter, resulting from events such as DNA repair deficiencies, cell cycle dysregulation, polymerase errors, and editing by APOBEC cytidine deaminases, provides cancer cells with growth advantages and evolutionary flexibility. This trait is a defining hallmark of cancer. However, genomic instability can also be a vulnerability for cancer cells. For instance, tumors with homologous recombination deficiency (HRD) are sensitive to PARP inhibitors that exacerbate DNA damage to an unsustainable level. Genomic instability also interacts intricately with the immune system. Mismatch repair deficiency (MMRD), which causes hypermutations, makes tumors susceptible to anti-PD-1 therapy. Similarly, the cGAS/STING pathway, activated by cytosolic DNA in tumors with genomic instability, can initiate immune responses. The clinical relevance of genomic instability, as exemplified by MMRD and HRD, underscores the need to assess tumors for such mechanisms. This is particularly important given that the clinical implications of several other types of genomic instabilities, including replication stress and APOBEC mutations, remain unclear.

Enhanced signature analysis

Mutational signature analysis identifies patterns corresponding to distinct biological processes, revealing a tumor's mutagenic history. However, current approaches frequently oversimplify the nature of mutagenesis by presuming linear accumulation and neglecting correlations both within mutational processes and their dependencies on global and tumor-specific topographical features. Through more realistic statistical modeling of DNA damage and repair processes, we develop new algorithms. By applying these methods to



We employ mutational signature analysis techniques to infer the origin of mutations, enabling us to categorize tumors based on their mechanisms of genomic instability. By leveraging large cancer genome datasets and using machine learning techniques, we create algorithms specifically designed for patient stratification in clinical settings to personalize their treatment. Part of this figure was created with BioRender.com.

rapidly growing datasets of whole-genome sequenced cancers, we aim to achieve a more detailed map of processes and improve the accuracy of genomic instability classification.

Dissecting the complexity

A significant challenge in the translation of signatures into clinical biomarkers is the pronounced diversity across the subtypes of tumors within a class of genomic instability. Consider APOBEC mutagenesis as an example: Based on the origin of single-stranded DNA, the mutations may occur on lagging strand in tumors with replication stress, the nontemplate strand in tumors with transcription stress, hairpins, DNA within micronuclei, or extrachromosomal DNA. Tumors that result from these distinct mechanisms are expected to demonstrate considerable variability in their molecular characteristics, which can limit the utility of signatures as biomarkers. Genomic diversity is not the only aspect to be considered; the relevance of genomic instability for treatments can differ based on the transcriptional profiles of tumors and the immune microenvironment, and these are highly tissue-specific. Moreover, as tumors evolve, all of these factors need to be monitored and reevaluated. Our lab aims to develop computational methods that can resolve these complexities, and tailored tools for clinical applications that can be used to guide cancer treatments.

Leveraging circulating tumor DNA

Circulating tumor DNA (ctDNA) offers a non-invasive means to capture the clonal and spatial heterogeneity as well as the temporal evolution of tumors. Mutational signature analyses using ctDNA have numerous potential clinical applications. For instance, they can be used to distinguish mutations of tumor origin from those due to clonal hematopoiesis or amplification artifacts. Consequently, they can play a particularly crucial role in development of strategies for early cancer diagnosis and evaluating minimal residual disease. We also construct signatures analysis algorithms tailored for ctDNA that can be used to classify patients non-invasively according to their genomic instability and monitor the changes in signatures which might signal development of resistance.

Selected Publications:

Jin H*, **Gulhan DC***, Ben-Isvy D, Geng D, Ljungstrom V, Park PJ. Accurate and sensitive mutational signature analysis with MuSiCal. *bioRxiv* 2022.04.21.489082.

Batalini F*, **Gulhan DC***, Mao V, Tran A, Polak M, Xiong N, Tayob N, Tung N, Winer EP, Mayer EL, Knappskog S, Mayer EL, Lønning PE, Matulonis UA, Konstantinopoulos PA, Solit DB, Won HH, Eikesdal HP, Park PJ, Wulf GM. Mutational signature 3 detected from clinical panel sequencing is associated with responses to olaparib in breast and ovarian cancers. *Clin. Cancer Res*. 2022 Nov 1;28(21):4714-4723.

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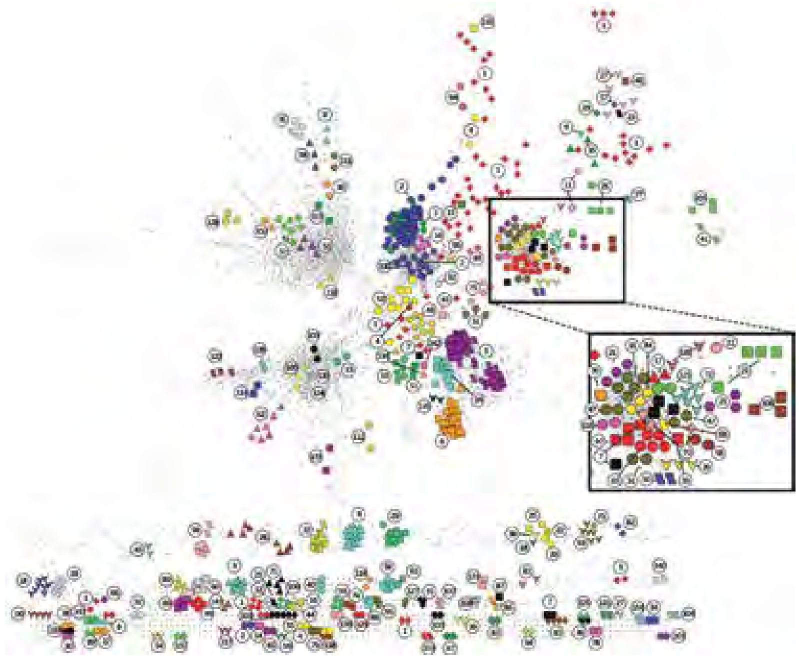
The Haas laboratory uses quantitative mass spectrometry-based proteomics to characterize cancer cells and their vulnerabilities in a comprehensive proteome-wide manner. This is fueled by recent discoveries that have enhanced the depth and throughput of proteomics in quantifying proteins and their post-translational 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. Another focus in the lab is to develop high-throughput plasma proteome mapping technologies to enable early detection of cancer across multiple cancer types in an unbiased manner. 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.

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 is accurately correlated across all analyzed cell lines. We showed that protein co-regulation analysis allows the genome-wide mapping



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

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 interactome dysregulation (DysReg) mapping. This novel method 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.

Our goals are to apply these 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.

We also recently identified the E3 ligase UBR4 as a key regulator in adjusting the concentration level of interacting proteins – the molecular mechanism enabling our interactome mapping – and we have shown that this role presents UBR4 as a target for treating aneuploid cancer.

Another goal of our group is to develop a novel high-throughput proteomics platform including an artificial intelligence (AI)-powered mass spectrometry data acquisition method to enable unbiased deep proteome mapping of plasma proteomes to enable early detection of cancer. Unbiased screening of more than 2000 plasma proteins (in 10 minutes per sample) rather than mapping a small of number of biomarkers will allow to enable a multi-biomarker assay for multiple cancer types that is constantly improved through adaptation to the detection accuracy.

Selected Publications:

Kathiresan M, Animesh S, Morris R, Kreuzer J, Patra KC, Shi L, Merritt J, Yin X, Benes CH, Bardeesy N, **Haas W**. Protein interactome homeostasis through an N-recognin E3 ligase is a vulnerability in aneuploid cancer. *bioRxiv*. 2023 May 4: 2023.05.04.539299.

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

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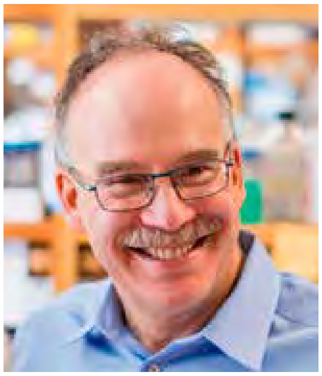

The Haber laboratory focuses on understanding mutations that are acquired by tumors and render them susceptible to specific targeted drug therapies. In 2004, we identified mutations in the EGFR gene in lung cancers which confer dramatic sensitivity to drugs that specifically inhibit that pathway. This finding triggered the application of targeted therapies in lung cancer, and more generally pointed to the critical importance of mutational analysis for treatment selection in common epithelial cancers. Since then, we have collaborated with the bioengineering team led by Dr. Mehmet Toner, the molecular biology group led by Dr. Shyamala Maheswaran, and the MGH Cancer Center clinical disease centers to develop, characterize and apply microfluidic devices to isolate rare circulating tumor cells (CTCs) in the blood of patients with cancer. Using these technologies, our lab seeks to explore 1) blood-based early detection of cancer, 2) noninvasive monitoring of cancer for the emergence of drug resistance, and 3) understanding mechanisms of tumor cell dissemination and metastasis, with the ultimate goal of suppressing blood-borne spread of cancer.

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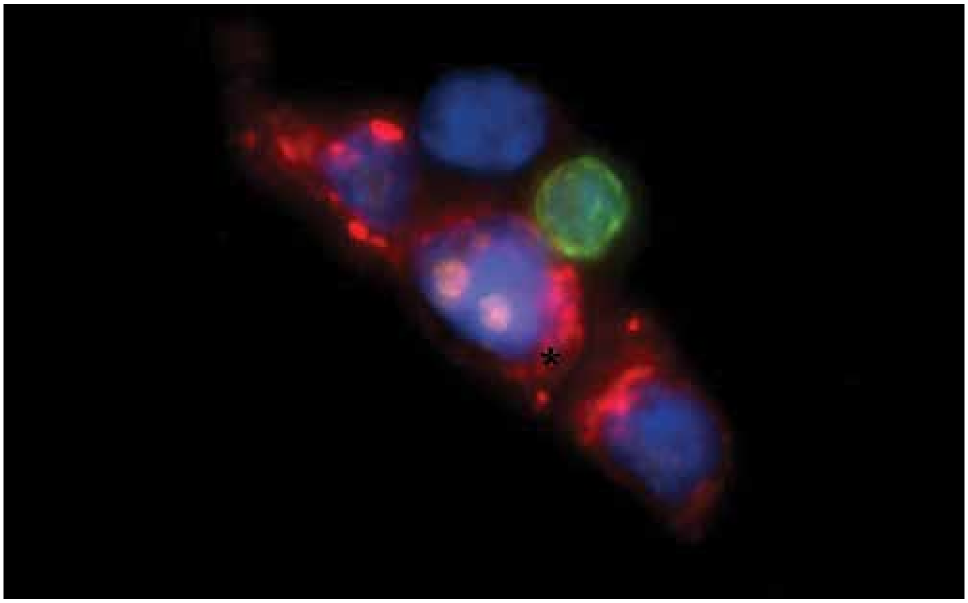
Our laboratory is interested in the genetics of human cancer. Current projects include the use of a microfluidic device to capture circulating tumor cells (CTCs) and its application in early detection of invasive cancer, molecular-directed therapy, and in the study of human cancer metastasis.

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 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



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

detection, we have we have applied high throughput CTC isolation from blood with molecular genetic and epigenetic markers.

Understanding metastasis through CTC biology

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 druggable therapeutic targets, such as *PIK3CA* and *FGFR*. In a recent study of prostate tumorigenesis, from the earliest Gleason stages through to metastatic CTCs, we tracked, at single cell level, core DNA hypomethylation domains that arise early in tumorigenesis, thereby silencing genes that are colocalized within a chromosomal locus. Early hypomethylation-induced silencing targets immune-related genes, notably the lipid antigen presentation pathway involved in native immunity, while sparing proliferation-associated genes. 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:

Guo H, Vuille JA, Wittner BS, Lachtara EM, Hou Y, Lin M, Zhao T, Raman AT, Russell HC, Reeves BA, Peskow HM, Wu CL, Meissner GA, Efstathiou JA, Lee RJ, Toner M, Aryee MJ, Lawrence MS, Miyamoto DT*, Maheswaran S*, **Haber DA***. DNA hypomethylation silences anti-tumor immune genes in early prostate cancer and CTCs. *Cell*, 186:2765-2782, 2023 PMID 37327786.

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Hong X, Roh W, Sullivan RJ, Wong KHK, Wittner BS,...Toner M, Stott SL, Getz G, Maheswaran S*, **Haber DA***. The lipogenic regulator SREBP2 induces transferrin in circulating melanoma cells and suppresses ferroptosis. *Cancer Discovery*. 11:678-95, 2021 PMID 33203734.

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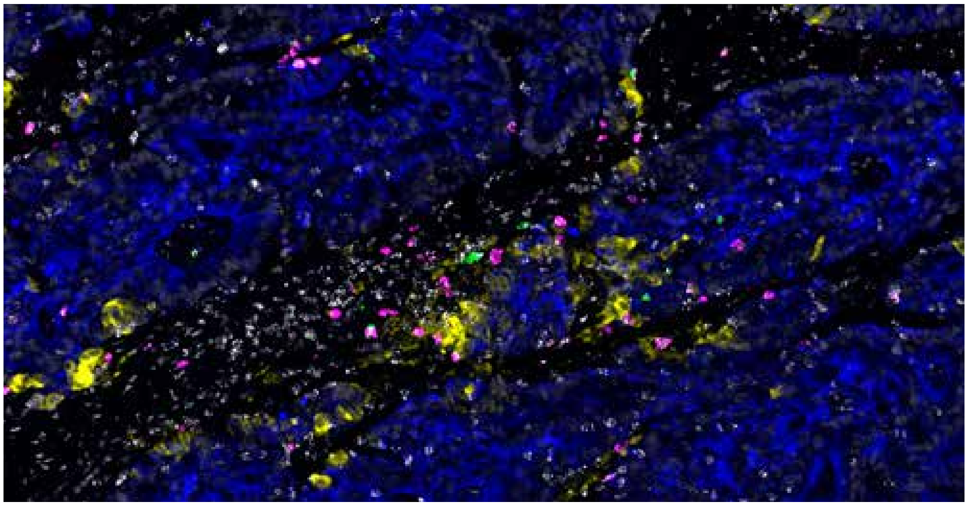
The Hacohen laboratory consists of immunologists, geneticists, biochemists, technologists, physicians and computational biologists working together to develop new and unbiased technologies and strategies to understand basic immune processes and immune-mediated diseases, with an emphasis on the innate immunity, tool development and personalized 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 performed 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 tumor genome.

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. 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've identified somatic mutations in tumors that are associated with anti-tumor immunity in patients, found T cell subtypes that are associated with a response to anti- PD-1 immunotherapy in melanoma and are studying their properties now (Sade-Feldman et al., *Cell* 2018), and discovered spatially-organized immune cell hubs in colon cancer (Pelka, Hofree, Chen et al, *Cell* 2021; Chen et al, *bioRxiv* 2023). We have also developed new methods to predict which tumor antigens are presented (Abelin et al., *Immunity* 2017, Sarkizova et al., *Nat Biotech* 2020), which are now being used to develop novel therapeutic approaches and targets for immunotherapy, such as personal tumor vaccines targeting multiple HLA-associated neoantigens in human tumors (together with Dr. Catherine Wu at DFCI, Ott et al., *Nature* 2017, Keskin 2018).

Genes and networks underlying innate immunity

We've used genome-wide CRISPR libraries to discover mammalian genes mediating the sensing of pathogens (Parnas et al., *Cell* 2015), impacting HIV infection (Park et al, *Nat Gen* 2017) and affecting influenza infection (Li et al., *Nat Comm* 2020). We have characterized innate myeloid cells (DCs and monocytes) in human blood as part of the human Immune Cell Atlas (Villani et al, *Science* 2017). We defined regulators of viral RNA-sensing (Carlson et al., *PNAS* 2023) and DNA-sensing pathways using FACS- and imaging-based screens. Recently, we discovered that the STING protein, a protein required for sensing cyclic di-nucleotides, is a proton channel that can trigger LC3B lipidation, inflammasome activation and cell death (Liu, Carlson et al., *Science* 2023).



In the subset of mismatch repair-deficient human colorectal tumors, activated and likely tumor-reactive T cells (white, green, and magenta) are organized into "hubs" around malignant cells (blue) expressing chemokines (yellow) that attract T cells and other cells into spatially organized immune cell hubs. Credit: Joshua Pirl, Vjola Jorgji, Linda Nieman, Jonathan Chen.

Source: Pelka, Hofree, Chen et al. Cell. 2021

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 characterized 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, and that genetic control of splicing is prevalent and important for the immune response (Ye et al., Genome Res 2018). Building on these studies, we developed systematic methods to analyze variants (Ray et al., *Nat Comm* 2021; Mouri, *Nat Genetics*, 2022). We also study non-genetic variations in human immunity, and found a myeloid cell type and state ('MS1' that corresponds to MDSCs) strongly associated with severe infections (bacterial and viral, including COVID-19) and sepsis (Reyes et al, *Nat Med* 2020, *Science Tr Med* 2021), leading us to new hypotheses underlying these dangerous clinical trajectories.

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. How does autoimmunity develop upon triggering of innate immunity by self DNA (rather than pathogen-derived DNA)? 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. To further find drivers of autoimmunity, we've been analyzing kidney biopsies and blood from lupus patients in a small (Arazi et al., *Nat Imm* 2019) and large patient cohort (ongoing) and more recently in comparison to animal lupus models.

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Liu B, Carlson RJ, Pires I, Gentili M, Feng E, Hellier Q, Schwartz MA, Blainey PC, Irvine DJ, **Hacohen N**. Human STING is a proton channel. *Science* 2023. Aug 4 ;381(6657):508-514.

Pelka K, Hofree M, Chen JH, Sarkizova S, Pirl JD, Jorgji V... Ng K, Giannakis M, Nieman LT, Boland GM, Aguirre AJ, Anderson AC, Rozenblatt-Rosen O, Regev A, **Hacohen N**. Spatially organized multicellular immune hubs in human colorectal cancer. *Cell*. 2021 Aug 24:S0092-8674(21)00945-4.

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Sade-Feldman M, Yizhak K, Bjorgaard SL, Ray JP, de Boer CG, Jenkins RW, Lieb DJ, Chen JH, Frederick DT, Barzily-Rokni M, Freeman SS...Cooper ZA, Paweletz CP, Barbie DA, Stemmer-Rachamimov S, Flaherty KT, Wargo JA, Boland GM, Sullivan RJ, Getz G and **Hacohen N**. Defining T cell states associated with response to checkpoint immunotherapy in melanoma. *Cell*. 2018 Nov 1;175(4):998-1013

Ott P, Hu X, Keskin DB, Shukla SA, Sun J, Bozbym DJ, Zhang W, Luoma A, Giobbie-Hurder A, Peter L, Chen C, Olive O, Carter TA, Li S, Lieb DJ, Eisenhaure T...Getz G, Wucherpfennig K, Neuberg D, Ritz J, Lander ES, Fritsch EF, **Hacohen N** & Wu CJ. An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature*. 2017 Jul 13;547(7662):217-221.

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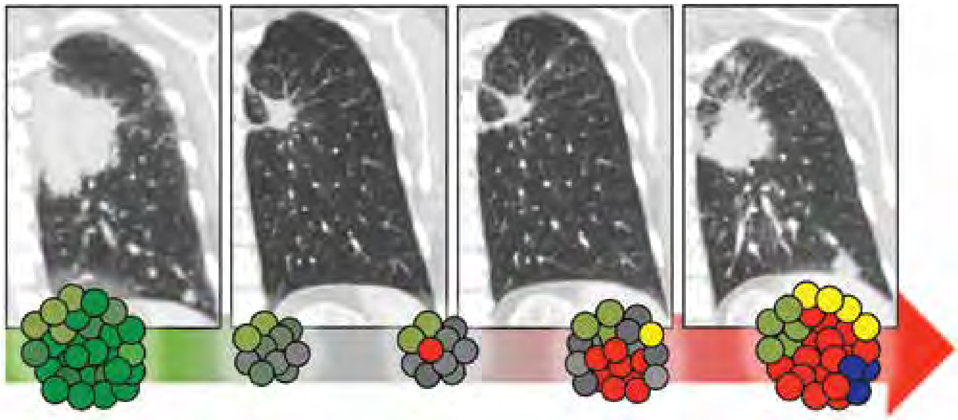
The research goal of **the Hata laboratory** is to advance the development of novel targeted and immunotherapy approaches to benefit patients with lung cancer. Our focus is on understanding biological mechanisms that dictate drug sensitivity and resistance in oncogene-addicted lung cancers (those with activating genetic alterations EGFR, ALK, KRAS, etc.). Our approach is highly translational, integrating assessment of clinical specimens with generation and analysis of patient-derived cell culture and mouse tumor xenograft (PDX) models, performed in close collaboration with clinicians in the MGH Thoracic Oncology group. We have discovered clinical mechanisms of acquired drug resistance and identified therapeutic strategies to overcome them. Our work has also shed light on how cancer cells adapt and evolve during the course of therapy and we are currently working to identify targetable vulnerabilities in cancer cells that can be exploited to prevent resistance from developing in the first place. Our ultimate goal is to translate our laboratory discoveries into clinical trials.

Mechanisms of acquired drug resistance to targeted therapies

Lung cancers that harbor activating EGFR mutations and ALK fusions are exquisitely sensitive to small molecule EGFR and ALK tyrosine kinase inhibitors, respectively. However, even though most patients experience dramatic responses, drug resistance invariably develops leading to disease relapse. Similar patterns of sensitivity and acquired resistance are also observed in other subsets of oncogene- addicted lung cancers treated with molecularly targeted therapies (e.g. ROS1 fusions, RET fusions, BRAF mutations, MET exon 14 skipping mutations). In collaboration with oncologists in the Mass General Center for Thoracic Cancers, we have identified acquired secondary mutations and other genomic alterations that cause drug resistance in the tumors and blood of patients progressing after initial response to targeted therapies. To functionally interrogate mechanisms of drug resistance, we have developed a robust infrastructure for generating patient-derived cell lines and mouse patient-derived xenograft (PDX) models from lung cancer patients treated with targeted therapies at the MGH Cancer Center. These models have enabled functional screens to identify novel mechanisms of acquired resistance and testing of novel next-generation therapies to overcome them.

Targeting KRAS mutant lung cancers

Mutant-selective KRAS inhibitors have recently entered the clinic, however responses are seen in only a minority of patients. Work by our group revealed that many KRAS mutant lung cancers exhibit decreased oncogenic dependency and a dampened apoptotic response that contributes to intrinsic resistance to KRAS targeted therapy. To overcome this limitation, we are exploring novel therapeutic combinations that can modify these mechanisms and increase sensitivity to KRAS inhibitors. In addition, we are focused on understanding how both inter-patient and intratumoral heterogeneity may influence initial drug response and clonal evolution, leading to the development of acquired drug resistance.



Oncogene-addicted lung cancers can develop acquired drug resistance by selection of pre-existing resistant cells, or via evolution of drug tolerant persister cells that subsequently develop resistance mechanisms during the course of treatment. Therapeutic strategies that eliminate persisters or block their ability to evolve may preempt the development of acquired drug resistance.

Tumor adaptation and evolution during treatment

Despite the development of successive generations of targeted therapies with improved selectivity and potency, acquired resistance inevitably develops. Our discovery that drug tolerant clones that survive initial therapy can acquire a "second genomic hit" enabling outgrowth of fully resistant clones suggests that these persister cells may comprise a cellular reservoir from which heterogeneous mechanisms of resistance may arise. We have identified that targeted therapies can induce expression of the cytodine deaminase APOBEC3A, which increases genomic instability and accelerates the development of drug resistance. Ongoing efforts are focused on characterizing persistent tumor cells in patients and experimental models to identify additional mechanisms that drive adaptation to drug, with the goal of to develop therapeutic strategies to preempt acquired drug resistance.

Impact of tumor microenvironment on drug response and resistance.

Non-cancer cells within the tumor microenvironment (TME), such fibroblasts and macrophages, can potentiate or attenuate drug response. We have uncovered a striking degree of complexity in functional interactions between cells in the TME that may contribute to heterogeneity of drug response in the clinic. By unraveling these mechanisms, we hope to develop orthogonal TME-centric therapeutic strategies to augment the effectiveness of currently approved targeted therapies.

Developing novel immunotherapy approaches for lung cancers with low mutation burden

EGFR mutant and ALK fusion lung cancers typically occur in never-smokers and consequently have low tumor mutation burden and poor response to currently approved immune checkpoint inhibitors. We are developing TCR cellular therapies and novel methods for reprograming tumor cell antigenicity to direct the immune system to recognize and fight EGFR and ALK lung cancers.

Selected Publications:

Isozaki H^, Sakhtemani R, Abbasi A, Nikpour N, Stanzione M, Oh S, Langenbucher A, Monroe S, Su W, Cabanos HF, Siddiqui FM, Phan N, Jalili P, Timonina D, Bilton S, Gomez-Caraballo M, Archibald HL, Nangia V, Dionne K, Riley A, Lawlor M, Banwait MK, Cobb RG, Zou L, Dyson NJ, Ott CJ, Benes C, Getz G, Chan CS, Shaw AT, Gainor JF, Lin JJ, Sequist LV, Piotrowska Z, Yeap BY, Engelman JA, Lee JJ, Maruvka YE, Buisson R, Lawrence MS*^, **Hata AN***^. Therapy-induced APOBEC3A drives evolution of persistent cancer cells. Nature. 2023 Aug;620(7973):393-401.

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Nangia V†, Siddiqui FM†,… Benes CH, Hughes PE, **Hata AN**. Exploiting MCL-1 dependency with combination MEK + MCL-1 inhibitors leads to induction of apoptosis and tumor regression in KRAS mutant non-small cell lung cancer. *Cancer Discovery*. 2018 Dec;8(12):1598-1613.

Yoda S, Lin JJ,…**Hata AN***, Shaw AT*. Sequential ALK Inhibitors Can Select for Lorlatinib-Resistant Compound ALK Mutations in ALK-Positive Lung Cancer. *Cancer Discovery*. 2018 Jun;8(6):714-729.

Hata AN†, Niederst MJ†,… Engelman, JA. Tumor cells can follow distinct evolutionary paths to become resistant to epidermal growth factor receptor inhibition. *Nature Medicine*. 2016; 22:262-9

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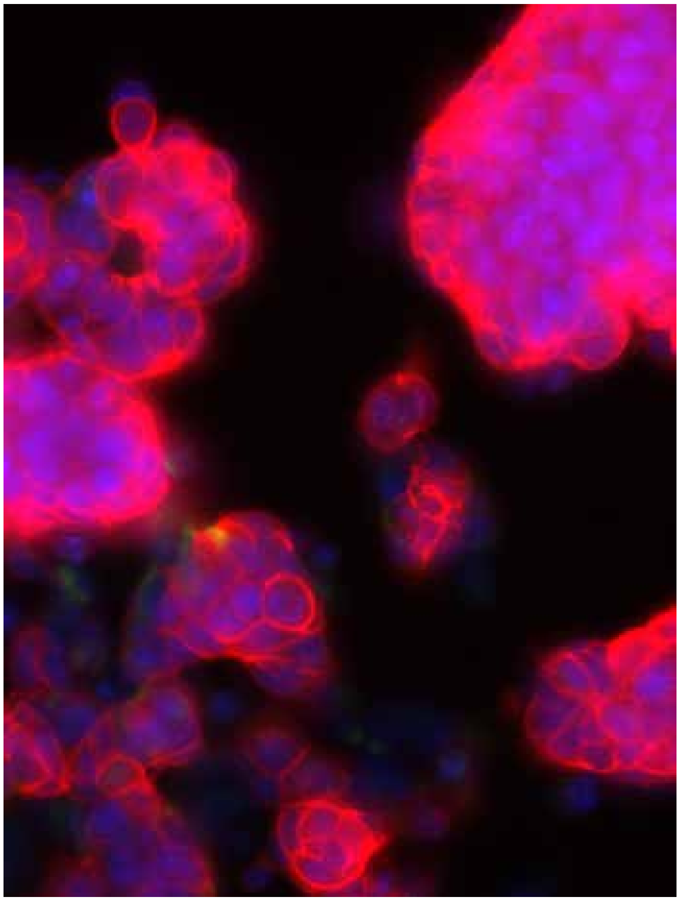
The Hochedlinger laboratory explores the fundamental question of how cells maintain their identity. We hypothesize that factors that reinforce specific cell states, such as pluripotency and differentiation, continue to play functional roles in other cellular contexts including development, tissue homeostasis and cancer. Using stem cell models and reprogramming systems as discovery tools ex vivo, our laboratory has elucidated novel mechanisms that maintain cell identity and function upstream of cell type specific transcription and chromatin factors. Specifically, work from our lab over the past five years revealed that common cellular processes such as protein sumoylation, chromatin assembly, alternative mRNA polyadenylation and P-body homeostasis play key roles in the maintenance of cell identity across distinct lineages. We now aim to probe the functional conservation of these mechanisms across physiological cell fate transitions in vivo using animal models and cell transplantation. As our strategy is not confined to one particular cell type or tissue, we are in a position to uncover shared regulatory principles crucial for the maintenance of cell identity across different developmental contexts.

While development and cellular differentiation were long thought to be irreversible processes, our ability to reprogram differentiated cells to an embryonic-like state revealed that mechanisms that safeguard cell identity and thus restrict developmental plasticity can be overcome through experimental manipulation. Indeed, seminal somatic cell nuclear transfer (SCNT) experiments proved that the nuclei of terminally differentiated cells and even certain cancer cells retain full developmental potential. While SCNT is a powerful assay to test the developmental potential of a given genome, it does not allow one to study *how* differentiated cell states are established and maintained. By contrast, transcription factor-induced reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) is a molecularly defined and tractable system to dissect fundamental questions of cell state. Our lab initially used this approach to provide crucial insight into the basic mechanisms by which transcription factors and chromatin signaling establish and maintain identity in either pluripotent or differentiated cells, and we began to probe the conservation of these principles in other cellular contexts. For example, we discovered that the transcription factor Sox2, which is essential for the establishment and maintenance of pluripotent stem cells, is re-expressed in adult gastric stem cells where it maintains tissue identity by suppressing an alternative intestinal cell program and tumorigenesis. Similarly, we demonstrated that the manipulation of safeguard mechanisms previously identified during iPSC reprogramming in other cellular contexts facilitate the derivation of self-renewing muscle stem-like cells, which have been notoriously difficult to capture using conventional strategies. Most recently, our lab uncovered two post-transcriptional processes, alternative polyadenylation (APA) and Processing body (P-body) turnover, as novel safeguard mechanisms using unbiased screens. While APA and P-bodies are thought to control different aspects of gene regulation in the nucleus (APA) and cytoplasm



Immunofluorescence images showing fibroblasts undergoing reprogramming to induced pluripotent stem cells upon forced expression of Oct4, Sox2, Klf4 and c-Myc in the presence of the histone mutant H3.3K36M. Note that cells express the epithelial marker Epcam (red) homogeneously but no longer express the fibroblast marker Vimentin (green), demonstrating that loss of H3K36 methylation is sufficient to endow the majority of somatic cells with an epithelial state that subsequently gives rise to iPSCs (See Hoetker et al., Nat Cell Bio, in press).

Image: Michael Hoetker, MD

(P-bodies), a key commonality that emerged from our work is that both processes regulate the protein homeostasis of hundreds of fate-instructive genes. Together, these examples underscore the power of our approach to gain insights into tissue identity through the study of pluripotency and cellular reprogramming.

Considering that several of the safeguard mechanisms we previously identified in reprogramming converge on chromatin regulators, we have recently developed versatile transgenic tools to directly probe the physiological role of chromatin modifications in cell fate change. This approach has allowed us to uncover previously unappreciated functions of H3K9 and H3K36 methylation in the regulation of pluripotency, reprogramming, tissue homeostasis and aging, which is the basis for ongoing work in the lab.

Thus, by pursuing our hypothesis that different physiological as well as experimentally induced cell fate transitions utilize common mechanisms, our lab has uncovered novel epigenetic, transcriptional and post-transcriptional regulators of cell identity. As we pursue a deeper understanding of how these underexplored regulators and processes guide cell fate transitions in vivo, we are poised to discover shared principles by which they safeguard cell identity during development and tissue homeostasis and how this knowledge may be exploited in a therapeutic setting to alter cell fate.

Selected Publications:

Hoetker, M. S., M. Yagi, B. Di Stefano, J. Langerman, S. Cristea, L. P. Wong, A. J. Huebner, J. Charlton, W. Deng, C. Haggerty, R. I. Sadreyev, A. Meissner, F. Michor, K. Plath, and K. **Hochedlinger K**. H3k36 Methylation Maintains Cell Identity by Regulating Opposing Lineage Programmes. *Nat Cell Biol* 2023 (in press)

Huebner, A. J., R. A. Gorelov, R. Deviatiiarov, S. Demharter, T. Kull, R. M. Walsh, M. S. Taylor, S. Steiger, J. T. Mullen, P. V. Kharchenko, and K. **Hochedlinger K**. Dissection of Gastric Homeostasis in Vivo Facilitates Permanent Capture of Isthmus-Like Stem Cells in Vitro. *Nat Cell Biol* 2023 (25), no. 3 (Mar): 390-403.

Yagi M, Ji F, Charlton J, Cristea S, Messemer K,…Goldhamer DJ, Wagers AJ, Michor F, Meissner A, Sadreyev RI, **Hochedlinger K**. Dissecting dual roles of MyoD during lineage conversion to mature myocytes and myogenic stem cells. *Genes Dev*. 2021 Sep 1;35(17-18):1209-1228.

Brumbaugh J, Kim IS, Ji F, Huebner AJ, Di Stefano B,…Meissner A, Sadreyev RI, Bernstein BE, Hock H, **Hochedlinger K**. Inducible histone K-to-M mutations are dynamic tools to probe the physiological role of site-specific histone methylation in vitro and in vivo. *Nat Cell Biol*. 2019 Nov;21(11):1449-1461.

Di Stefano B, Luo EC, Haggerty C,… Gygi SP, Sadreyev RI, Meissner A, Yeo GW, **Hochedlinger K**. The RNA Helicase DDX6 Controls Cellular Plasticity by Modulating P-Body Homeostasis. *Cell Stem Cell.* 2019 Nov 7;25(5):622-638.e13.

Brumbaugh J, Di Stefano B, Wang X,…Elledge SJ, Chen Y, Sadreyev RI, Gygi SP, Hu G, Shi Y, **Hochedlinger K**. Nudt21 Controls Cell Fate by Connecting Alternative Polyadenylation to Chromatin Signaling. *Cell*. 2018 Jan 11;172(1-2):106-120.e21.

Hanno Hock, MD, PhD

Hock Laboratory

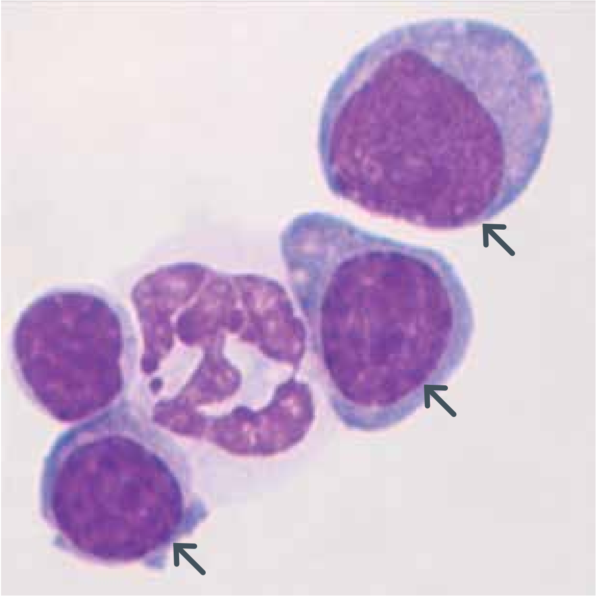
Hanno Hock, MD, PhD
Daniel Kramer
Ondrej Krejci, PhD
Ryan LeGraw

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.

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



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.

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.

Selected Publications:

Nardi V, Ku N, Frigault MJ, Dubuc AM, Tsai HK, Amrein PC, Hobbs GS, Brunner AM, Narayan R, Burke ME, Foster J, Dal Cin P, Maus MV, Fathi AT, **Hock H**.Clinical response to larotrectinib in adult Philadelphia chromosome-like ALL with cryptic ETV6-NTRK3 rearrangement. *Blood Adv*. 2020;4(1):106-11.

Brumbaugh J, Kim IS, Ji F, Huebner AJ, Di Stefano B, Schwarz BA, Charlton J, Coffey A, Choi J, Walsh RM, Schindler JW, Anselmo A, Meissner A, Sadreyev RI, Bernstein BE, **Hock H***, Hochedlinger K*. Inducible histone K-to-M mutations are dynamic tools to probe the physiological role of site-specific histone methylation in vitro and in vivo. *Nat Cell Biol*. 2019;21(11):1449-61.

Hock H, and A. Shimamura. 2017. ETV6 in hematopoiesis and leukemia predisposition. *Seminars in hematology* 54:98-104. PMC5584538

Foudi A, Kramer DJ, Qin J, Ye D, Behlich AS, Mordecai S, Preffer FI, Amzallag A, Ramaswamy S, Hochedlinger K, Orkin SH and **Hock H**. Distinct, strict requirements for Gfi-1b in adult bone marrow red cell and platelet generation. *J Exp Med*. 2014; 211, 909–927.

Qin J, Whyte WA, Anderssen E, Apostolou E, Chen H, Akbarian S, Bronson RT, Hochedlinger K, Ramaswamy S, Young RA, and **Hock H**. The Polycomb Group Protein L3mbtl2 Assembles an Atypical PRC1-family Complex with Essential Roles in Pluripotent Stem Cells and Early Development. *Cell Stem Cell*. 2012; 11, 319-332, 2012.

Schindler JW, Van Buren D, Foudi A, Krejci O, Qin J, Orkin SH, **Hock H**. TEL-AML1 corrupts hematopoietic stem cells to persist in the bone marrow and initiate leukemia. *Cell Stem Cell*. 2009; Jul 2, 5(1):43-53.

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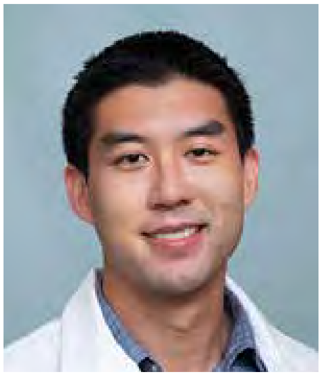
William L. Hwang, MD, PhD



The Hwang laboratory focuses on the immense phenotypic, temporal and spatial heterogeneity of tumor ecosystems and the many insights that can only be gleaned by studying these systems at the level of their individual components — single molecules or cells. We study tumor-stroma interactions at unprecedented resolution through the development and application of techniques in spatial and systems oncology, advanced microscopy, genetic engineering and computational biology to patient-derived specimens, stromal tumoroids and mouse models. Our goals are to elucidate mechanisms of (1) therapeutic resistance mediated by genetic, epigenetic, and phenotypic factors including cell state plasticity; (2) treatment-mediated remodeling of the spatial microarchitecture of tumors and underlying cancer cell-stromal interactions; and (3) tumor-nerve-immune crosstalk, which plays a critical role in the pathophysiology and morbidity of many malignancies but remains understudied.

Hwang Laboratory

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Single-cell dynamics

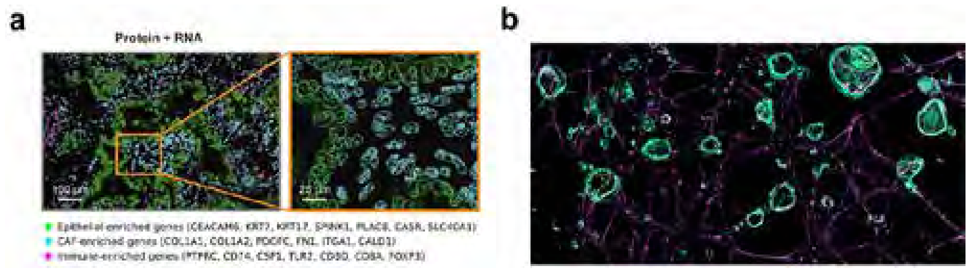
Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal and treatment refractory disease. Molecular subtyping of PDAC is rudimentary and does not currently inform clinical management or therapeutic development. We optimized single-nucleus RNA-seq to discover treatment-associated changes in cellular composition and state, including enrichment of a novel neural-like malignant program in residual tumors after chemoradiation. Our high-resolution molecular framework elucidates the inter- and intra-tumoral diversity of PDAC, treatment-associated remodeling and clinically relevant prognostication to enable precision oncology in PDAC.

Ongoing projects:

1. Identifying key regulators, context dependence and therapeutic vulnerabilities of resistant cell states
2. Elucidating (epi)genetic contributions to cell state plasticity in therapeutic resistance
3. Investigating mechanisms of tumorigenesis using single-cell multiomics to enable chemoprevention and early detection
4. Studying developmental lineages and mechanisms of metastasis in pancreatic neuroendocrine tumors

Spatial oncology

Dissociative single-cell approaches enable detailed characterization of the different cell types and states that compose a heterogeneous tumor but sacrifice in situ spatial relationships among cells. Leveraging recent advances in spatial proteo-transcriptomics enabling single-cell resolution and high molecular plex, we performed spatial molecular profiling (SMI) on a cohort of patient-derived PDAC tumors and developed a novel method for inferring multicellular interactions. Spatially Constrained Optimal Transport Interaction Analysis (SCOTIA) that considers both spatial distance and ligand-receptor (LR) expression (collaborator: Martin Hemberg). We used SCOTIA to dissect the remodeled pancreatic tumor microenvironment in response to neoadjuvant chemoradiation and uncovered marked changes in LR interactions between cancer-associated fibroblasts and malignant cells, which was supported by orthogonal experiments using a murine tumoroid co-culture system (*https://tinyurl.com/2xtdytxt*).



(a) Spatial coordinates of RNA transcripts for canonical epithelial (green), CAF (cyan) and immune (magenta) marker genes, overlaid on an immunofluorescence image from spatial molecular imaging (SMI). Inset depicts a magnified region with cell segmentation boundaries (cyan). (b) 3D co-culture of genetically-engineered cancer organoids (green) with sensory neurons (magenta) in a Matrigel dome.

Overall, we demonstrated the immense potential of a translational spatial biology paradigm for deriving novel biological insights and identifying actionable therapeutic targets — one that can be broadly applied to other malignancies and treatment contexts.

Ongoing projects:

1. Discovering gene regulatory networks that modulate tumor-stroma interactions through perturbative spatial screens
2. Developing computational models to infer cell state from integrating intrinsic and extrinsic influences
3. Creating a platform for correlating morphological changes to transcriptional changes through combining live-cell imaging with spatial transcriptomics
4. Integrating matched liquid and spatial biomarkers to assess response to therapy

Cancer neuroscience

Active recruitment of nerve fibers into tumors plays an important role in cancer development, treatment resistance, metastasis and mortality for many malignancies, but the diverse molecular mechanisms underlying tumor-nerve crosstalk remain largely unknown. To address this gap in knowledge, we performed a comprehensive, cell-type specific, spatially resolved whole transcriptome analysis of human PDAC using custom tissue microarrays derived from intratumorally matched malignant areas with (N+) and without (N-) nerve involvement. Whole-transcriptome digital spatial profiling revealed that classical malignant cells were depleted near nerves while basal/mesenchymal and neural-like cancer cells were enriched near nerves. Differential gene expression analysis comparing malignant cells in N+ versus N- regions enabled selection of subtype-specific candidate genes for functional investigation. This research will provide a detailed understanding of the mechanisms by which pancreatic cancer cells and the peripheral nervous system collaborate to confer numerous pro-tumorigenic effects, and guide prioritization for therapeutic intervention in the burgeoning cancer neuroscience field.

Ongoing projects:

1. Identifying cell-type specific mediators of nerve outgrowth, invasion and colonization using patient-derived tumors, tumoroids and GEMMs
2. Determining influence of neuronal subtype and activity on the immune response to cancer in primary tumors and draining lymph nodes
3. Dissecting molecular mechanisms of dynamic physical interactions between cancer cells and nerves
4. Discovering the mechanistic basis for differential central nervous system versus peripheral nervous system tropism across the spectrum of cancer

Selected Publications:

Hwang WL*, Jagadeesh KA*, Guo JA*, Hoffman HI*, Yadollahpour P, Reeves J, … Fernandez-del Castillo C, Liss AS, Ting DT, Jacks T†, Regev A‡. Single-nucleus and spatial transcriptome profiling of pancreatic cancer identifies multicellular dynamics associated with neoadjuvant treatment. *Nature Genetics* 2022 Aug;54(8):1178-1191.

Shi DD, Guo JA, Hoffman HI, Su J, Mino-Kenudson M, Barth JL, Schenkel JM, Loeffler JS, Shih HA, Hong TS, Wo JY, Aguirre AJ, Jacks TJ, Zheng L, Wen PY, Wang TC, **Hwang WL**†. Therapeutic avenues for cancer neuroscience: translational frontiers and clinical opportunities. *Lancet Oncology*. 2022;23(2):e62-74.

Gu, JA, Hoffman HI, Weekes CD, Zheng LZ, Ting DT, **Hwang WL**†. Refining the molecular framework for pancreatic cancer with single-cell and spatial technologies. *Clinical Cancer Research*. 2021;27(14):3825-3833.

Guo JA, Hoffman HI, Shroff S, Chen P, Hwang PG, Kim DY, Kim DW, Cheng SW, Zhao D, Mahal BA, Alshalafa M, Niemierko A, Wo JY, Loeffler JS, Fernandez-del Castillo C, Jacks T, Aguirre AJ, Hong TS, Mino-Kenudson M, **Hwang WL**†. Pan-cancer transcriptomic predictors of perineural invasion improve occult histopathological detection. *Clinical Cancer Research*. 2021;27(10):2807-2815.

Hwang WL*, Pike LRG*, Royce TJ, Mahal BA, Loeffler JS†. Safety of combining radiotherapy with immune checkpoint inhibitors. *Nature Reviews Clinical Oncology.* 2018;15(8):477-94.

Hwang WL*, Deindl S*, Harada BT, Zhuang X†. Histone H4 tail mediates allosteric regulation of nucleosome remodeling by linker DNA. *Nature*. 2014;512(7513):213-7.

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A. John Iafrate, MD, PhD

Iafrate Laboratory

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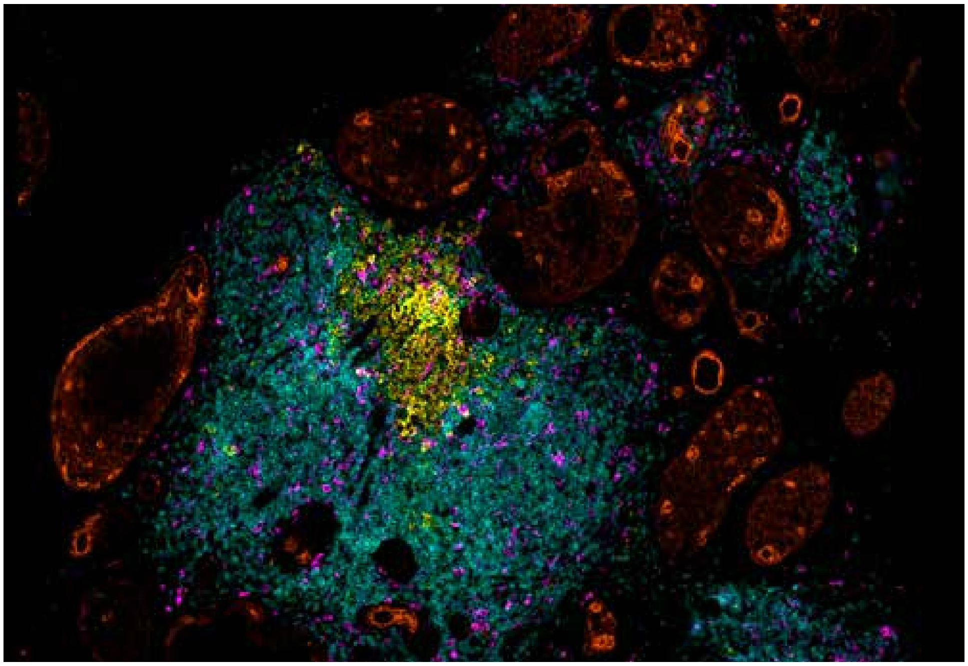
The Iafrate laboratory has focused efforts on developing highly complex molecular analyses of tumor genetics using novel technologies. We have a strong interest in the clinical implementation of genetic screening technologies that can help direct targeted therapies, focusing on lung, breast and brain tumors. Our recent contributions in the treatment of a subset of non-small cell lung carcinoma (NSCLC) with rearrangements of the ALK tyrosine kinase, rearrangements of the ROS1 tyrosine kinase and MET exon 14 skipping with a small molecule kinase inhibitor (crizotinib), underscore the promise of personalized cancer care (1, 2). We currently are focusing on detecting tumor DNA in blood samples ("liquid biopsies") to allow for efficient and convenient tracking of cancer progression. In additional we are developing new techniques to allow for early detection of cancers by detecting tumor-specific DNA in circulation.

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 (3). 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. We have also initiated studies of tumor heterogeneity; these efforts focus on gene amplification of receptor tyrosine kinases in glioblastoma (4). 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 (5), see image. 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.

More recently we have adapted the AMP sequencing technology 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. Using AMP we have developed tissue-specific cell-free DNA (cfDNA) panels to examine the most important cancer genes in common tumors, including lung,



Multiplex Immunofluorescence to detect changes in the immune landscape in head and neck tumors.

melanoma, breast and colon cancer. Such panels are allowing us to track, with a simple blood draw, the tumor burden in patients. We are able to use cfDNA analysis in patients with metastatic cancer to see if they are responding to therapy, and also can track the development of resistance mutations. This allows a real-time dynamic optimization of therapy. Most recently we have developed a methylation-based sequencing assay to allow efficient analysis of tumor-specific methylation patterns in cfDNA samples. We hope that such an approach can be a lot more sensitive in the detection of small amounts of circulating tumor DNA, allowing potential early detection of tumors before they are clinically symptomatic. In addition, the methylation patterns are actually specific to the type of tumor the DNA is derived from, potentially allowing us to determine the actual anatomic site of origin.

The lab has developed multiplex immunofluo rescence panels to study the spatial biology of tumor types including ovarian cancer (looking at homologous recombination repair proteins) and head and neck cancan (looking at immune infiltrates).

Using the Lunaphore platform, the lab can simultaneously examine >15 markers at true singles cell resolution. We have development computation pipelines to analyze these complex datasets.

Selected Publications:

Garcia-Beltran WF, lab EC, Astudillo MG, Yang D, Miller TE, Feldman J, Hauser BM, Caradonna TM, Clayton KL, Nitido AD, Murali MR, Alter G, Charles RC, Dighe A, Branda Ja, Lennerz JK, Lingwood D, Schmidt AG, **Iafrate AJ**, Balazs AB. Covid-19-neutralizing antibodies predict disease severity and survival. *Cell*. 2021; 21;184(2):476-488.

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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.

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Othon Iliopoulos, MD

Iliopoulos Laboratory

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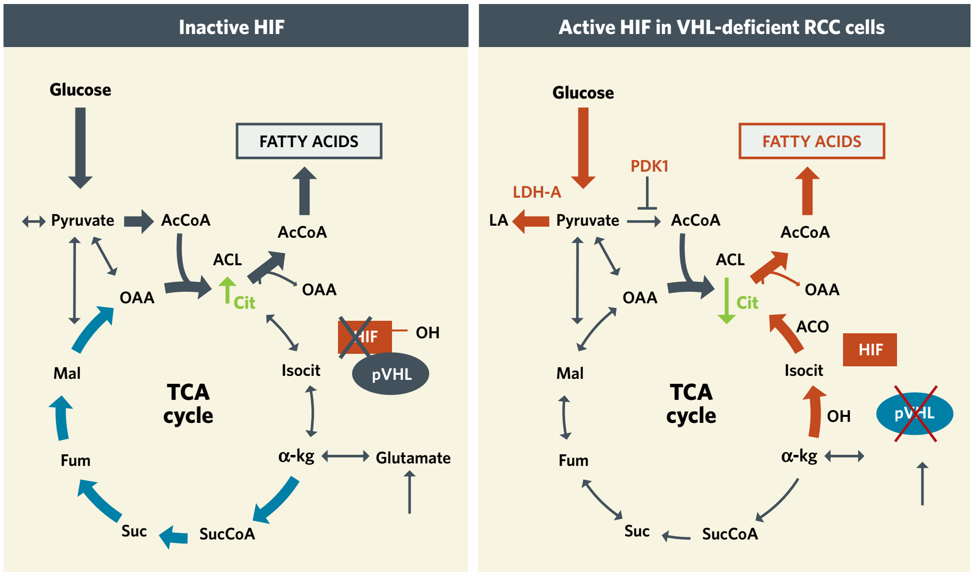
The Iliopoulos laboratory works on the main mechanisms underlying the reprogramming of cancer cell metabolism and cancer angiogenesis with the goal to develop mechanism-based strategies for selectively killing cancer cells. We use Renal Cell Carcinoma (RCC) as a model disease of altered cancer metabolism 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.

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.

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).



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

We brought these fundamental observationsof our laboratory on glutamine metabolism to the clinic, testing the combination of GLS1 inhibitors with PARP inhibitors in renal cancer, clear cell ovarian and prostate cancer

Clinical and translational studies to identify resistance to the HIF2a inhibitor Belzutifan.

Belzutifan has been approved by FDA for treatment of VHL disease related RCC, hemagioblastoma and pancreatic neuroendocrine tumors. Our laboratory and the MGH VHL and Hemangioblastoma Centers are leading clinical trials for the optimal use of this first in class oral medication. In addition, we use patient tissue, in vitro and in vivo models to discover mechanisms of resistance to this medication.

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. Targeting metabolism in RCC. *Nature Reviews Nephrology*. 2017; 13, 320.

Laviolette LA, Mermoud J, Calvo IA, Olson N, Boukhali M, Steinlein OK, Roider E, Sattler EC, Huang D, Teh BT, Motamedi M, Haas W, **Iliopoulos O**. Negative regulation of EGFR signalling by the human folliculin tumour suppressor protein. *Nat Commun*. 2017; 28;8: 15866.

Metelo AM, Noonan HR, Li X, Jin YN, Baker R, Kamentsky L, Zhang Y, van Rooijen E, Shin J, Carpenter AE, Yeh JR, Peterson RT, **Iliopoulos O.** Treatment of VHL disease pheno-types with small molecule HIF2a inhibitors. *Journal Clinical Investigation*. 2015; 125 (5):1987-97.

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.

Metallo CM, Gameiro PA, Bell EL, Mattaini KR, Yang J, Hiller K, Jewell CM, Zachary R. Johnson JR, Irvine DJ, Guarente G, Kelleher JK, Vander Heiden MG, **Iliopoulos O***, Stephanopoulos G*. Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. *Nature*. 2011; 481 (7381): 380-4, Nov 20.

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.

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The Jan laboratory primarily focuses on the development of clinically suitable synthetic biology platforms in order to advance next-generation cellular immunotherapies. Harnessing elegant protein degradation cellular machinery that has evolved to control fast biologic transitions related to information flow and signal processing, we have developed molecular switch technologies regulated by the FDA-approved drug lenalidomide as generalizable chemical biology tools and cell therapy controllers. We use genomics, synthetic biology, and biochemistry to build new technologies, explore design principles for adaptive, user-controllable immune cells, and investigate clinical settings to deploy smart cell therapies.

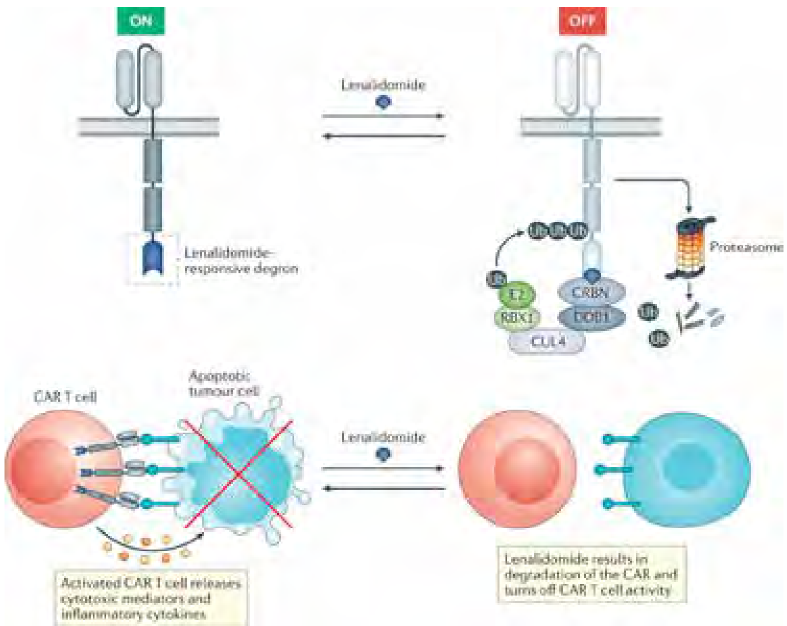
Programming cellular immunotherapies using targeted protein degradation

Genetically modified (CAR) T cells have emerged as transformative agents in the care of people with cancer. To reach their full potential, cellular immunotherapies must become safer, more effective, and more accessible. Mentored by Drs. Marcela Maus and Benjamin Ebert, we recently developed chemical genetic controls systems around the FDA-approved drug lenalidomide and its analogs, which act as molecular glue targeted protein degraders, recruiting neosubstrate proteins to E3 ubiquitin ligases for polyubiquitination and proteasomal degradation. We engineered clinically suitable lenalidomide-inducible dimerization and degradation systems, and with them drug ON- and OFF-switch CAR T cells (see Figure). We are now exploring specific scenarios where control over the dynamics of CAR signaling can mitigate T cell hyperactivation toxicities and allow for higher potency designs. These inducible degradation systems have also been further leveraged to encode additional functions in investigational cellular immunotherapies. To tune up the anti-tumor potency of CAR T cells, we have developed chemical genetic cytokine delivery systems, enabling spatiotemporally controlled release of potent T cell proliferative and anti-tumor cytokine signals that have a poor therapeutic window when delivered systemically. For highly potent and/or novel investigational cell therapies with unproven safety profiles, together with the Manguso lab, we are developing cell therapy suicide switches induced by lenalidomide that may act as safeguards in early-stage clinical testing.

We have also developed a new technology to genetically reprogram E3 ubiquitin ligases to bind and degrade customizable sets of endogenous proteins. This system for targeted endogenous protein degradation in engineered cells can act constitutively, in response to a small molecule controller drug, or in integrated sense-and-response synthetic circuits. Using this protein-protein interaction-based molecular logic for post-translational endogenous protein regulation, we are exploring diverse applications to engineer new and therapeutically useful functions not only in T cells but also in NK cells and hematopoietic stem cells.

Design and evaluation of cellular immunotherapies targeting novel antigens

CAR T cells can be highly effective and well-tolerated therapeutics when they are targeting antigens that are homogenously expressed on tumor cells and are also



Molecular switch control of genetically engineered cell therapies. *Incorporation of a lenalidomide-responsive degron tag enables drug-dependent degradation mediated by the ubiquitin-proteasome system. Pharmacologic control can be used to mitigate CAR T cell hyperactivation toxicities or to tune CAR signaling. Image credit: Nature Reviews Clinical Oncology.* *Image credit: Nature Reviews Clinical Oncology.*

absent from essential normal tissues. In collaboration with the Villani lab, we are leveraging single cell genomics and large-scale tumor and normal tissue gene expression datasets to nominate novel target antigens in select solid tumors. In collaboration with the Manguso lab and others, we are leveraging innovative approaches to engineer affinity reagents for tumor sensing by CAR T cells, here applied to target a founding, clone-specific surface neoantigen in a subtype of myeloproliferative neoplasm. In the long term, we seek to integrate novel tumor antigen discovery and fit-for-purpose molecular logic systems into investigational cellular immunotherapies targeting malignancies with limited treatment options.

Understanding anti-tumor T cell fate and plasticity using dynamic perturbations

Having developed a suite of tools, including small molecule-controllable genome editing proteins, that can be used in primary human T cells for fast and reversible perturbations of target genes and proteins, we seek to understand how dynamic perturbations can shape and even reprogram T cell fate and function. Transient and traceable perturbations may enable the study of stage-specific molecular mechanisms governing T cell lineage and differentiation trajectories, as well as nascent therapeutic opportunities leveraging rapid development of targeted in vivo delivery modalities.

Selected Publications:

Sreekanth V, **Jan M**, Zhao KT, Lim D, Davis JR, McConkey M, ... & Choudhary A. A molecular glue approach to control the half-life of CRISPR-based technologies. *bioRxiv*. 2023 Mar 20:2023.03.

Bouzid H, Belk JA, **Jan M**, Qi Y, Sarnowski C, Wirth S, ... & Jaiswal S. Clonal hematopoiesis is associated with protection from Alzheimer's disease. *Nature Medicine*. 2023 Jul;29(7):1662-1670.

Lane IC, & **Jan M**. SEAKER cells coordinate cellular immunotherapy with localized chemotherapy. *Trends in Pharmacological Sciences.* 2022 Oct;43(10):804-805.

Jan M*, Sperling AS*, & Ebert BL. Cancer therapies based on targeted protein degradation—lessons learned with lenalidomide. *Nature Reviews Clinical Oncology*. 2021 Jul;18(7):401-417.

Jan M, Scarfò I, Larson RC, Walker A, Schmidts A, Guirguis AA, ... Maus MV, & Ebert BL. Reversible ON-and OFF-switch chimeric antigen receptors controlled by lenalidomide. *Science Translational Medicine*, 2021 Jan 6;13(575):eabb6295.

Jan M, Leventhal MJ, Morgan EA, Wengrod JC, Nag A, Drinan SD, ... & Ebert BL (2019). Recurrent genetic HLA loss in AML relapsed after matched unrelated allogeneic hematopoietic cell transplantation. *Blood Advances*., 2019 Jul 11;134(2): 160-170.

*Equal contribution

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.

Jenkins Laboratory

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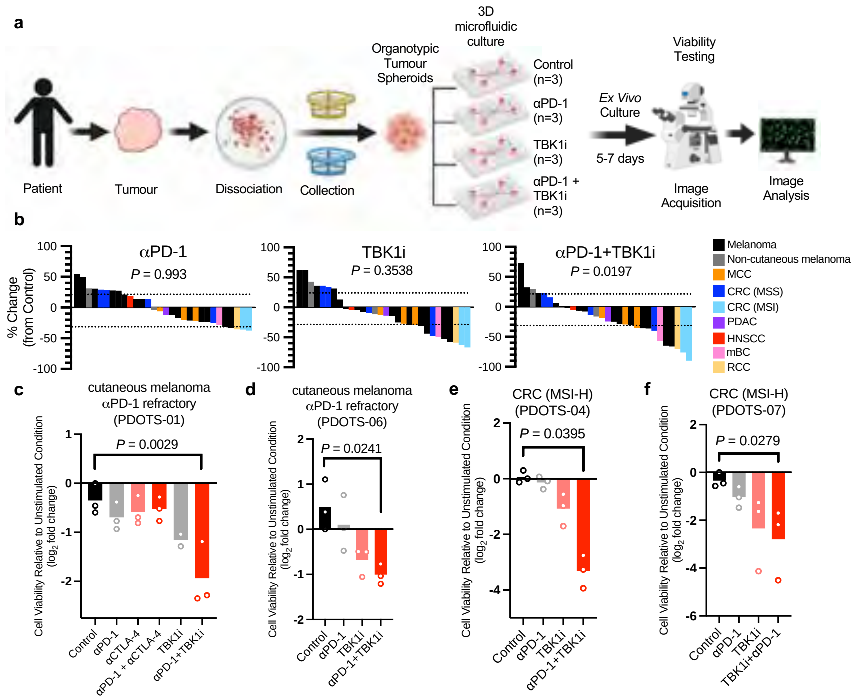
Precision cancer medicine currently focuses on knowledge of the cancer mutation repertoire and the tailored application of drugs that target altered genes or pathways in individual patients, such as use of BRAF inhibitors in patients with BRAF mutant melanoma. Immune checkpoint inhibitors targeting the 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.

We demonstrated that organotypic tumor spheroids isolated from fresh mouse and human tumor samples retain autologous lymphoid and myeloid cell populations, including antigen- experienced tumor



TBK1 inhibition enhances sensitivity to PD-1 blockade using PDOTS. a, Schematic of PDOTS preparation. b, Waterfall plots for PDOTS (n = 30, indicated tumor types) treated with anti-PD-1 (250 µg/ml pembrolizumab), TBK1i (1 µM) or combined anti-PD-1 + TBK1i. Mean values (bars) for each sample are shown. Statistical analysis was performed using one-way ANOVA (matched) with Dunnett's multiple- comparison test compared with the control. MCC, Merkel cell carcinoma; CRC, colorectal cancer; MSS, microsatellite stable; PDAC, pancreatic ductal adenocarcinoma; HNSCC, head and neck squamous cell carcinoma; mBC, metastatic breast cancer; RCC, renal cell carcinoma.(ref: Sun et al., Nature 2023)

infiltrating CD4 and CD8 T lymphocytes, and respond to PD-1 blockade in short-term *ex vivo* culture (Jenkins et al., *Cancer Discovery* 2018; PMID: 29101162).

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.

Recently, we identified the innate immune kinase TANK-binding kinase 1 (*TBK1*) as a candidate immune-evasion gene in a pooled genetic screen. Using a suite of genetic and pharmacological tools across multiple experimental model systems, we confirm a role for *TBK1* as an immune-evasion gene. Targeting TBK1 enhances responses to PD-1 blockade by decreasing the cytotoxicity threshold to effector cytokines (TNF and IFNγ). TBK1 inhibition in combination with PD-1 blockade also demonstrated efficacy using patient-derived tumor models, with concordant findings in matched patient-derived organotypic tumor spheroids and matched patient-derived organoids. Tumor cells lacking TBK1 are primed to undergo RIPK- and caspase- dependent cell death in response to TNF and IFNγ in a JAK–STAT-dependent manner. Taken together, our results demonstrate that targeting TBK1 is an effective strategy to overcome resistance to cancer immunotherapy.

Selected Publications:

Sun Y, Revach O-Y, Anderson S, Kessler EA, Wolfe CH, Jenney A, et al. Manguso RT, **Jenkins RW**. Targeting TBK1 to overcome resistance to cancer immunotherapy. *Nature*. 2023;615:158–67.

Revach OY, Liu S, **Jenkins RW**. Targeting TANK-binding kinase 1 (TBK1) in cancer. *Expert Opin Ther Targets*. 2020 Nov;24(11):1065-1078.

Sade-Feldman M, Yizhak K, Bjorgaard SL, Ray JP, de Boer CG, **Jenkins RW**, Lieb DJ, Chen JH, Frederick DT, Barzily-Rokni M, Freeman SS, Reuben A, Hoover PJ, Villani AC, Ivanova E, Portell A, Lizotte PH, Aref AR, Eliane JP, Hammond MR, Vitzthum H, Blackmon SM, Li B, Gopalakrishnan V, Reddy SM, Cooper ZA, Paweletz CP, Barbie DA, Stemmer-Rachamimov A, Flaherty KT, Wargo JA, Boland GM, Sullivan RJ, Getz G, Hacohen N. Defining T Cell States Associated with Response to Checkpoint Immunotherapy in Melanoma. *Cell*. 2018 Nov 1;175(4):998-1013.

Aref AR, Campisi M, Ivanova E, Portell A, Larios D, Piel BP, Mathur N, Zhou C, Coakley RV, Bartels A, Bowden M, Herbert Z, Hill S, Gilhooley S, Carter J, Cañadas I, Thai TC, Kitajima S, Chiono V, Paweletz CP, Barbie DA, Kamm RD, **Jenkins RW**. 3D microfluidic ex vivo culture of organotypic tumor spheroids to model immune checkpoint blockade. *Lab Chip*. 2018 Oct 9;18(20):3129-3143.

Jenkins RW, Aref AR, Lizotte PH, et al. Ex Vivo Profiling of PD-1 Blockade Using Organotypic Tumor Spheroids. *Cancer Discov*. 2018;8(2):196-215.

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.

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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 drug targets and therapies to treat relapse and refractory disease.

Identifying molecular pathways that drive progression and relapse in pediatric cancer

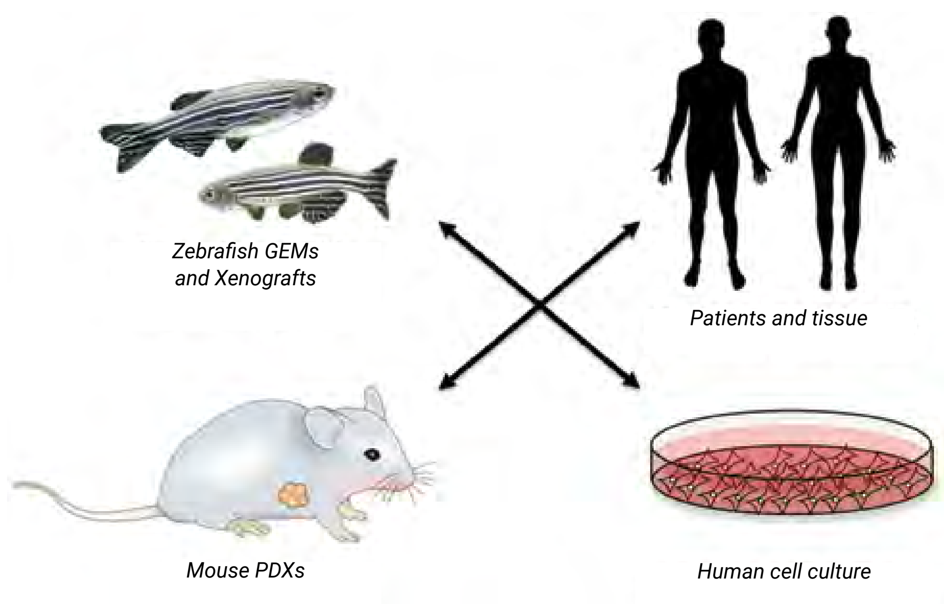
The Langenau laboratory uses zebrafish genetic models, human cell lines, patient derived xenografts, and patient samples to uncover progression and relapse mechanisms in pediatric T-cell acute lymphoblastic leukemia (T-ALL) and rhabdomyosarcoma (RMS) muscle cancer. Our work has detailed the remarkable conservation of molecular mechanisms in zebrafish and human cancer and discovered novel biology and new therapies for these diseases. For example, we identified combination Olaparib and temozolomide therapy for the treatment of RMS that is in clinical trial evaluation for RMS patients at Mass General and Dana-Farber Cancer Institute in Boston (NCT01858168, Yan et al., *Cell* 2019).

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, high-throughput cell transplantation, and unbiased bioinformatic approaches, we have uncovered new oncogenic drivers associated with aggression, therapy resistance and relapse. A large subset of these genes exerts important roles in regulating human T-ALL proliferation, apoptosis and response to therapy. Discovering new relapse-driving oncogenic pathways will likely identify drug targets for the treatment of T-ALL.

Cancer stem cell pathways in pediatric muscle cancer

Rhabdomyosarcoma is a common soft-tissue sarcoma of childhood and phenotypically recapitulates fetal muscle development arrested at early stages of differentiation. Our laboratory has developed transgenic zebrafish models of RMS that mimic the molecular underpinnings of human disease to discover functionally-distinct cell subpopulations, including cancer stems that drive continued tumor growth at relapse. Remarkably these same cell states are found in human disease and drive therapy resistance (Wei et al, *Nature Cancer* 2022). Our group has also uncovered important roles for WNT, MYOD transcription factors, the VANGL2/non-canonical WNT pathway, NOTCH, and P53 loss in driving continued RMS growth.



The Langenau lab uses a wide array of cancer models to discovery new mechansims of progression and relapse. Genetically-engineered models (GEMs) and patient-derived xenografts (PDXs).

Zebrafish avatars of human cancer

The Langenau Lab has generated a number of immunocompromised zebrafish strains that efficiently engraft human tumors. These models are amenable to real-time imaging of cancer hallmarks at single cell resolution and have been used in preclinical modeling experiments to identify drug combinations and new immunotherapy approaches for the treatment of human rhabdomyosarcoma and other cancers. This work has led to the first clinical trial for pediatric cancer originating from findings made in the zebrafish.

Selected Publications:

Wei Y, Qin Q, Yan C, Hayes MN, Garcia SP, Xi H, Do D, Jin AH, Eng TC, McCarthy KM, Adhikari A, Onozato ML, Spentzos D, Neilsen GP, Iafrate AJ, Wexler LH, Pyle AD, Suvà ML, Dela Cruz F, Pinello L, **Langenau DM**. Single-cell analysis and functional characterization uncover the stem cell hierarchies and developmental origins of rhabdomyosarcoma. *Nat Cancer*. 2022;3(8):961-975.

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Yan C, Yang Q, Zhang S, Millar DG, Alpert EJ, Do D, Veloso A, Brunson DC, Drapkin BJ, Stanzione M, Scarfo I, Moore J, Iyer S, Qin Q, Wei Y, McCarthy KM, Rawls JF, Dyson NJ, Cobbold M, Maus M, **Langenau DM**. Single cell imaging of T cell immunotherapy responses in vivo. *J Exp Med*. 2021; 218(10):e20210314.

Patton EE, Zon LI, **Langenau DM**. Zebrafish disease models in drug discovery: from preclinical modelling to clinical trials. *Nat Rev Drug Discov*. 2021;20(8):611-628.

Yan C, Brunson DC, Tang Q, Do D, Iftimia NA, Moore JC, Hayes MN, Welker AM, Garcia EG, Dubash TD, Hong X, Drapkin BJ, Myers DT, Phat S, Volorio A, Marvin DL, Ligorio M, Dershowitz L, McCarthy KM, Karabacak MN, Fletcher JA, Sgroi DC, Iafrate JA, Maheswaran S, Dyson NJ, Haber DA, Rawls JF, **Langenau DM**. Visualizing Engrafted Human Cancer and Therapy Responses in Immunodeficient Zebrafish. *Cell*. 2019;177(7):1903-1914.

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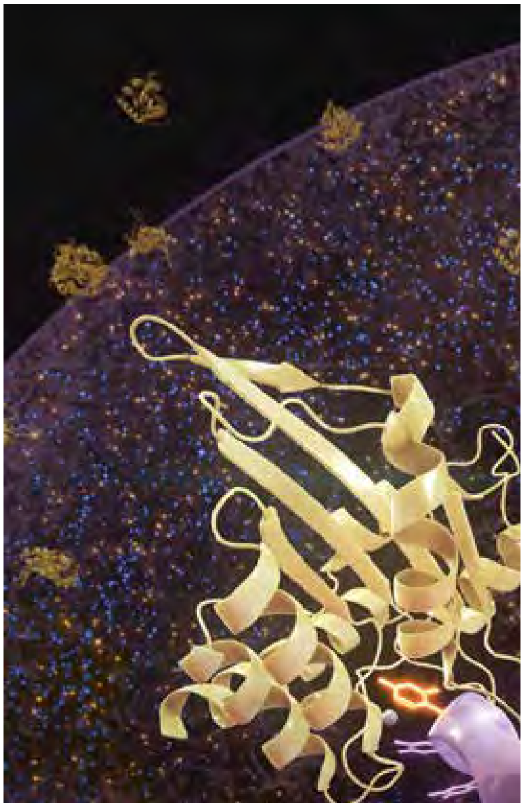
Cancer results from alterations to DNA that lead to the activation of oncogenes or the inactivation of tumor suppressors. **The Lawrence laboratory** focuses on understanding the many ways this can happen, using computation as a powerful microscope to study the processes of DNA damage and repair, gene 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.

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 mutations 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 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.

APOBEC mutations and mesoscale genomic features

Statistical approaches for distinguishing driver mutations from passenger mutations have relied on the gold standard of recurrence across patients. Seeing exactly the same DNA base-pair mutated recurrently across patients has been taken as proof that the



The mutational landscape of a cancer cell across size regimes. At the smallest scale, local DNA trinucleotide sequences (lower-left foreground) correlate with the "mutational signatures" induced by various mutagens. At the largest scale (background of image), chromatin is organized into multi-megabase domains comprising Compartment B (tightly packed, gene-poor DNA lining the nuclear periphery) and Compartment A (gene-rich open DNA in the nuclear interior). Mutations induced by APOBEC enzymes (yellow points) are distributed equally across the two compartments, but most other types of mutations (blue points) are concentrated in Compartment B. Between the large and small extremes lies the "mesoscale" regime, where genomic features like hairpin-forming ability are determined. DNA exposed in a hairpin loop is vulnerable to attack by the enzyme APOBEC3A (center), giving rise to highly recurrent passenger mutations in cancer.

mutation must be under functional selection for contributing to tumor fitness. The assumption is that mutational processes, being essentially random, are unlikely to hit the exact same base-pair over and over again. Our recent discoveries about APOBEC mutagenesis have cast doubt on this assumption. We have shown that APOBEC3A has a very strong preference for mutating cytosines presented in a short loop at the end of a strongly paired DNA hairpins. Our results indicate that there are multiple routes to cancer mutational hotspots. Driver mutation hotspots in oncogenes can rise to prominence through positive selection, and are not restricted to the "favorite" sites of any particular mutagen. In contrast, special DNA sites (like hairpins) that happen to be optimal substrates for a mutagen (like APOBEC) can give rise to "passenger hotspot mutations" that owe their prevalence to substrate optimality, not to any effects on tumor fitness. These findings apply not just to APOBEC but to all mutation signatures, and remind us of the need to be careful about assuming that all recurrent mutations are causative drivers of disease.

Selected Publications:

Isozaki H^, Sakhtemani R, Abbasi A, Nikpour N, Stanzione M, Oh S, Langenbucher A, Monroe S, Su W, Cabanos HF, Siddiqui FM, Phan N, Jalili P, Timonina D, Bilton S, Gomez-Caraballo M, Archibald HL, Nangia V, Dionne K, Riley A, Lawlor M, Banwait MK, Cobb RG, Zou L, Dyson NJ, Ott CJ, Benes C, Getz G, Chan CS, Shaw AT, Gainor JF, Lin JJ, Sequist LV, Piotrowska Z, Yeap BY, Engelman JA, Lee JJ, Maruvka YE, Buisson R, Lawrence MS*^, **Hata AN***^. Therapy-induced APOBEC3A drives evolution of persistent cancer cells. Nature. 2023 Aug;620(7973):393-401.

Langenbucher A, Bowen D, Sakhtemani R, Bournique E, Wise JF, Zou L*, Bhagwat AS*, Buisson R*, **Lawrence MS***. An extended APOBEC3A mutation signature in cancer. *Nat Commun*. 2021 Mar 11;12(1):1602.

Jalili P, Bowen D, Langenbucher A, Park S, Aguirre K, Corcoran RB, Fleischman AG, **Lawrence MS***, Zou L*, Buisson R*. Quantification of ongoing APOBEC3A activity in tumor cells by monitoring RNA editing at hotspots. *Nat Commun*. 2020 Jun 12;11(1):2971.

Buisson R, Langenbucher A, Bowen D, Kwan EE, Benes CH, Zou L*, **Lawrence MS***. Passenger hotspot mutations in cancer driven by APOBEC3A and mesoscale features. *Science*. 2019 Jun 28; 364(6447):eaaw2872.

Buisson R, **Lawrence MS**, Benes C, Zou L. APOBEC3A and APOBEC3B activities render cancer cells susceptible to ATR inhibition. *Cancer Res*. 2017 Jul 11.

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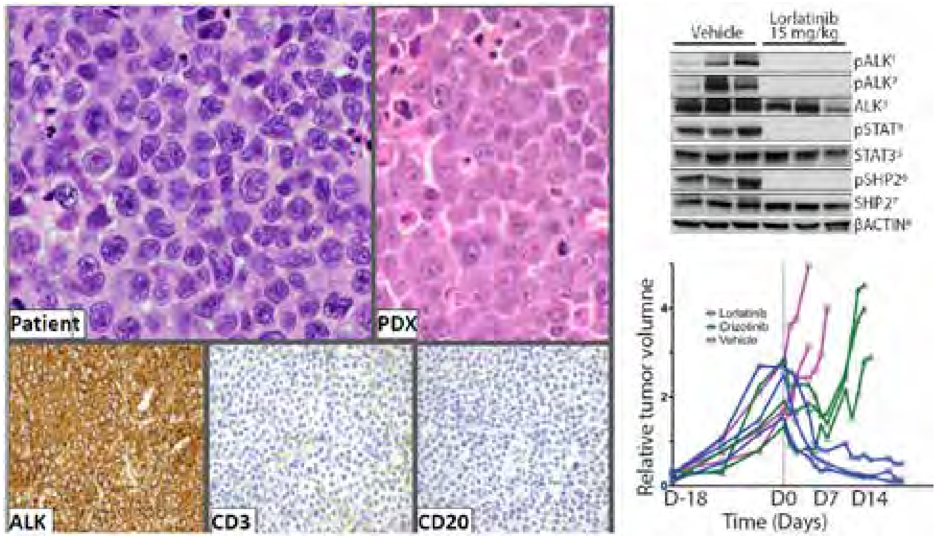
The Louissaint laboratory is interested in understanding how intrinsic genetic alterations and interactions of the lymphoma microenvironment drive lymphoma biology and determine the distinctive clinical behaviors of different lymphoma types. As part of our efforts, we aim to identify biomarkers of prognosis and responsiveness to therapy and to discover potential novel therapeutic targets that may be translated into improved outcomes for lymphoma patients. Traditionally, such investigation has been limited by the paucity of *in-vitro* and *in-vivo* models that faithfully capture the genetic and functional heterogeneity of human lymphomas. To overcome this challenge, our laboratory creates novel in-vivo patient-derived xenograft models and in-vitro primary cell models of lymphoma to investigate the role of genetic alterations, intratumoral heterogeneity, and microenvironment in lymphoma pathogenesis and to test the efficacy of specific therapeutic agents.

Defining novel therapeutic vulnerabilities in aggressive subtypes of large B-cell lymphoma

There are several aggressive lymphoma subtypes of B-cell lineage for which effective therapies do not exist and for which clinical trials sometimes cannot be performed due to the rarity of the diseases and the rapidity with which patients succumb to disease. Some of these lymphomas characterized by plasmablast phenotype do not respond well to standard B-cell chemotherapies and have particularly poor prognosis. One example, anaplastic lymphoma kinase (ALK)-positive large B-cell lymphoma (ALK-LBCL), is characterized by the abnormal expression of alkaline phosphatase protein (ALK), resulting from the production of an abnormal fusion gene of CLTC with ALK. Patients who acquire this lymphoma are typically young and have a dismal prognosis — often dying within two years of diagnosis after failed attempts with standard chemotherapy regimens and preliminary efforts with first generation ALK inhibitors.

We recently created the first patient-derived xenograft (PDX) models of ALK-LBCL that recapitulates the phenotypes and molecular features of the patient lymphomas. Using these xenograft models, we showed that next-generation ALK inhibitors (ALKi) (alectinib and lorlatinib) are active in ALK-LBCL, while the first generation crizotinib inhibitors are not. In collaboration with clinical colleagues, we translated these findings to patients in a multi-institutional study in which advanced stage, chemotherapy refractory ALK-LBCL patients were treated with alectinib followed by allogeneic transplantation, resulting in the first long-term remissions reported in this disease. We have recently developed primary *in-vitro* models of ALK+ LBCL that we are currently using in functional studies to further understand the pathobiological mechanisms driven by ALK fusions in this disease and to identify novel downstream vulnerabilities to complement ALKi therapies, as well as to define the unique mechanisms underlying ALK inhibitor resistance in this disease. We are also actively working on other similarly aggressive molecular subtypes of plasmablastic-type lymphomas and poor-prognosis molecular subtypes of diffuse large B-cell lymphoma using *in-vivo* and *in-vitro* models created in our laboratory.



Efficacy of ALK inhibitors (ALKi) in patient derived xenograph (PDX) models of ALK+ Large B-cell lymphoma. The image on the left shows the histology and immunophenotype of the PDX. The Western (upper right) show activity of ALKi (Lorlatinib) on ALK phosphorylation and signaling in the PDX tumor. The figure (lower right) shows efficacy of third-generation ALKi Lorlatinib on PDX ALK+ LBCL tumor (in contrast to transient partial response to first-generation ALKi Crizotinib).

Unraveling the role of the tumor microenvironment in follicular lymphoma

Follicular lymphoma (FL) is the second most common non-Hodgkin lymphoma, accounting for approximately one quarter of new cases worldwide. As the quintessential indolent B-cell lymphoma, FL is an incurable disease characterized by multiple relapses and frequent transformation (t-FL) to more aggressive lymphomas. Approximately 20% of patients requiring chemotherapy at diagnosis show early progression, usually associated with poor outcomes.

FL, like other indolent B-cell lymphomas, is comprised of heterogeneous population of malignant B cells within a prominent tumor microenvironment including various T cell populations, follicular dendritic cell and other stromal cell populations and some myeloid populations. Interactions between these malignant B cells and elements of tumor microenvironment are critical for FL to thrive. We aim to understand the role of these interactions in lymphoma pathogenesis, and in driving early progression of disease, with the goal of possibly targeting these mechanisms therapeutically.

A major impediment to answering these questions has been the lack of *in-vivo* and *in-vitro* models of human disease that can recapitulate the complexity of genetic alterations and cellular interactions between FL clones and microenvironment that define these lymphomas. We are creating patient-derived xenograft models and *in-vitro* primary models of follicular lymphoma for the purpose of studying these critical cellular interactions within the tumor microenvironment. To unravel and dissect these critical interactions, we are applying single cell sequencing technologies, together with powerful new single cell resolution multi-modal spatial genomics technologies in collaboration with colleagues Vignesh Shanmugam, Fei Chen and Todd Golub. These efforts will accelerate our understanding of the interplay of genetic alterations and microenvironment in driving the biology of indolent lymphomas and drive the discovery of novel targets of these diseases.

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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 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 heterogeneous cancer cell populations to metastasis and their significance as biomarkers and therapeutic targets.

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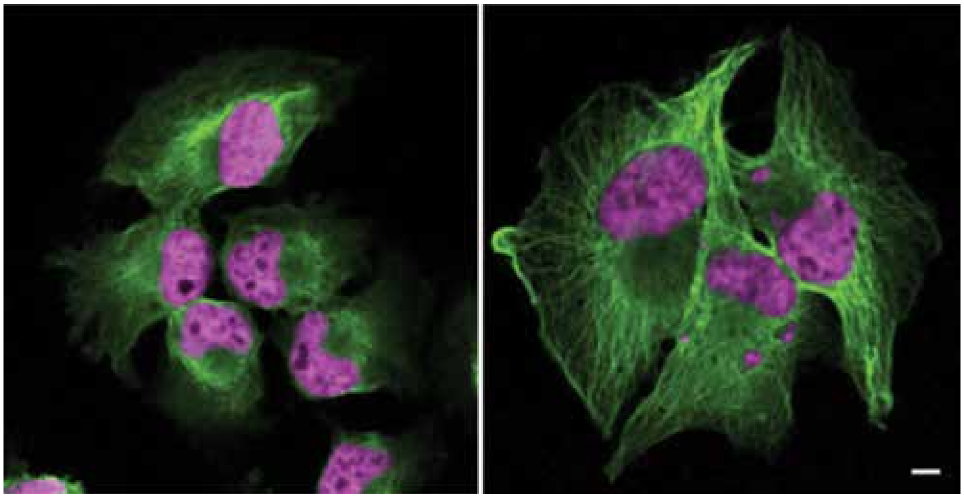
Mechanisms of Breast Cancer Metastasis

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, 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 to cellular states including: epithelial to mesenchymal plasticity, senescence, and how these aspects of tumor heterogeneity influence cancer progression and therapeutic responses.

Naturally occurring senescence induced by microenvironmental factors

Senescence is associated with the secretion of bioactive molecules - the senescence-associated secretory phenotype (SASP). SASP, which is context dependent, remodels the cellular microenvironment and contributes to many age-related diseases. Senolytic compounds, that eliminate senescent cells, alleviate these age-related conditions in preclinical models and in clinical trials; thus, senescence is a druggable cell state. TGFß, prevalent in the hypoxic tumor microenvironment, induces senescence in cancers, rendering it a physiological tumor cell state. In an immune-competent mouse lung cancer model, suppressing TGFß signaling, specifically in the tumor cells, ablated senescent cells in tumors and mitigated immune suppressive immune infiltration. In a therapeutic setting, non-small cell lung cancers with high TGFß/hypoxia-signaling and increased senescence - exhibit poor progression-free survival upon receiving immune checkpoint inhibitors shGFP escape shSETD1A escape



Confocal images of cells stained with tubulin (green) and DAPI (magenta) show that SETD1A-KD cells escaping senescence harbor chromosome segregation defects visualized as micronuclei (circled). The scale bar represents 50 μm.

(ICI). We are now exploring whether microenvironmental hypoxia-TGFß-induced physiological senescence and SASP are exploited by tumors to mount an innate resistance to ICIs, and how we can exploit this phenotype to improve ICI responses.

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The Manguso laboratory is working to improve the efficacy of cancer immunotherapy. We use a range of approaches including mouse models, functional genomics, cellular immunology, and single-cell profiling to understand how cancers evade the immune system. Our lab has pioneered the use of *in vivo* genetic screens with CRISPR to identify new immunotherapy targets and resistance mechanisms. Using these approaches, we identified the tyrosine phosphatase PTPN2, a critical regulator of immunotherapy sensitivity in tumor cells. We also identified the dsRNA-editing enzyme ADAR1 as a checkpoint that regulates the sensing of self-dsRNA by tumor cells. Our results indicate that there are dozens of ways that cancers can be targeted by the immune system, and we are working to understand the new mechanisms revealed by our studies. In the long term, these approaches will enable a new understanding of how the immune system interacts with cancerous tissue and how the interaction can be manipulated to destroy tumors.

Over the last decade, critical discoveries in immunology and cancer biology have revealed how tumors are shaped by the immune system and how they evolve to evade it. We now know that disrupting immune checkpoints such as CTLA-4 and PD-1/PD-L1 can lead to T cell-mediated elimination of tumors. However, there is still a critical unmet need, as the vast majority of patients with cancer do not benefit from current immunotherapies. Our most pressing challenge is to discover the next generation of immunotherapies that can bring clinical benefit to the majority of patients.

To discover immunotherapy targets and resistance mechanisms in high throughput, we have developed an *in vivo*, CRISPR-based genetic screening system to identify genes that regulate tumor cell sensitivity to immunotherapy (Manguso et al, *Nature* 2017). We genetically modify mouse cancer cell lines that can be transplanted into animals and used as immunotherapy models. After delivery of Cas9 and libraries of single guide RNAs (sgRNAs), we implant pools of modified tumor cells into animals that are treated with immunotherapy. In a single experiment we can determine genes that, when deleted, increase or decrease sensitivity to immunotherapy (Figure 1). This strategy has enabled the rapid and simultaneous identification of new targets and resistance mechanisms that are potent regulators of anti-tumor immunity.

This powerful, unbiased discovery system allows us to identify targets and resistance mechanisms with no previously identified roles in immunotherapy. Three examples illustrate the power of this system for discovery: 1) we found that deletion of the phosphatase PTPN2 enhanced tumor cell sensitivity to immunotherapy. While PTPN2 was known to negatively regulate T cell receptor activation, our screens determined that it is also the most potent suppressor of interferon-gamma sensing in tumor cells; 2) we discovered that the non-classical MHC-I gene HT-T23/Qa-1 (HLA-E) is a major immune checkpoint that limits anti-tumor immunity by T cells and NK cells; 3) our screens identified that deletion of ADAR1, an adenosine deaminase acting

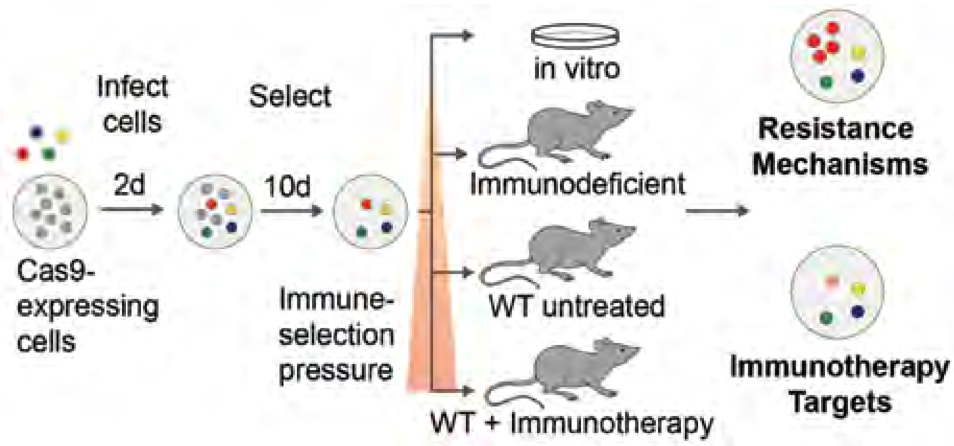

Diagram of in vivo CRISPR screening system. Pools of Cas9-expressing, sgRNA library-transduced tumor cells are implanted into either wild-type or immunocompromised mice. After 2 weeks, tumors are harvested and genomic DNA is extracted from tumor tissue. Next generation sequencing of the sgRNA library is used to identify resistance mechanisms or immunotherapy targets.

on RNA unmasks endogenous dsRNA that can be recognized by the cytosolic pattern recognition receptors PKR and MDA5, and can overcome resistance to immunotherapy caused by loss of antigen presentation (Ishizuka & Manguso et al, *Nature* 2019). Previously, these genes were not known or prioritized targets in immuno-oncology, but our unbiased approach enables discoveries that would have otherwise been unlikely.

We have demonstrated that *in vivo* CRISPR screens are a powerful way to discover new targets and probe the interaction of tumor cells with the host immune system. We can now broadly apply these genetic tools to advance our understanding of how immunotherapy works, why it may fail, and how we can improve it. Ongoing projects in the lab include:

1. Discover novel immunotherapy targets and mechanisms of resistance across several well-characterized mouse cancer models
2. Identify pathways that can overcome acquired resistance to immunotherapy
3. Understand how we can manipulate antigen presentation to enhance immunotherapy

These projects will define new ways to generate anti-tumor immune responses, reveal pathways that can be targeted to enhance these responses across cancer types, and anticipate and overcome the mechanisms by which tumors will become resistant. More broadly, these studies will improve our understanding of how tumors evolve under the selective pressure of immune surveillance and enable the development of more effective therapeutics.

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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 cure 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.

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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. Our laboratory focuses on T cell biology and T cell engineering. We design chimeric antigen receptors (CARs) 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 B cell malignancies such leukemia and lymphoma. 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 T cells as immunotherapy in patients with cancer.

The Mass General 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 test genetically-modified T cells in human subjects, and a "reverse translation" arm to examine the engraftment, persistence, and bioactivity of T cell products infused into patients. The immune profiling of patients is performed by the Immune Monitoring Laboratory, directed by Dr. Kathleen Gallagher.

Specifically, the engineered T cells that the Maus lab generates are intended to overcome specific obstacles observed in the clinic. The next generation T cells will:

1. *Contain molecular designs to enhance specificity, potency, and safety.*

   Novel types of antigen receptors are in development to target multiple antigens on tumor cells, which improves elimination of heterogenous tumor cells and prevents antigen-negative relapse while also decreasing the risk of targeting healthy cells. We are also using novel techniques to improve CAR T cell safety by regulating their activation and the molecules they release when activated. In liquid tumors, the focus is on improving the safety of CAR T cells, while in solid tumors, the focus is increasing their potency.

CAR-T Cell Targeting a Glioblastoma Cell Expressing EGFRvIII, Scanning Electron Micrograph; Credit: Bryan D. Choi, Mark B. Leick, and Marcela V. Maus.

2. *Be administered in combination with other drugs to sensitize tumors to T cell mediated killing and/or potentiate T cell function.*

   Many of the small molecule drugs and antibodies used in the clinic exert their effects on signaling pathways in tumor cells, T cells, and other immune cells. We aim to discover synergistic drug/T cell combinations to increase safety and efficacy, and use genetic engineering tools to confer specific drug sensitivity, resistance, or enhanced molecular switches.

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 or cytokines 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 microenvironment.

4. *Further build on the basic biology and mechanisms that drive natural and engineered T cell functions.*

   We aim to understand the signaling mechanisms and effector functions used by CAR T cells versus native T cells, to further improve CAR T cell efficacy and safety. By understanding how CAR T cells kill tumor cells, we can also decipher how tumors cells become or are intrinsically resistant to killing by CAR T cells. We can then better engineer CAR T cells to prevent resistance from occurring.

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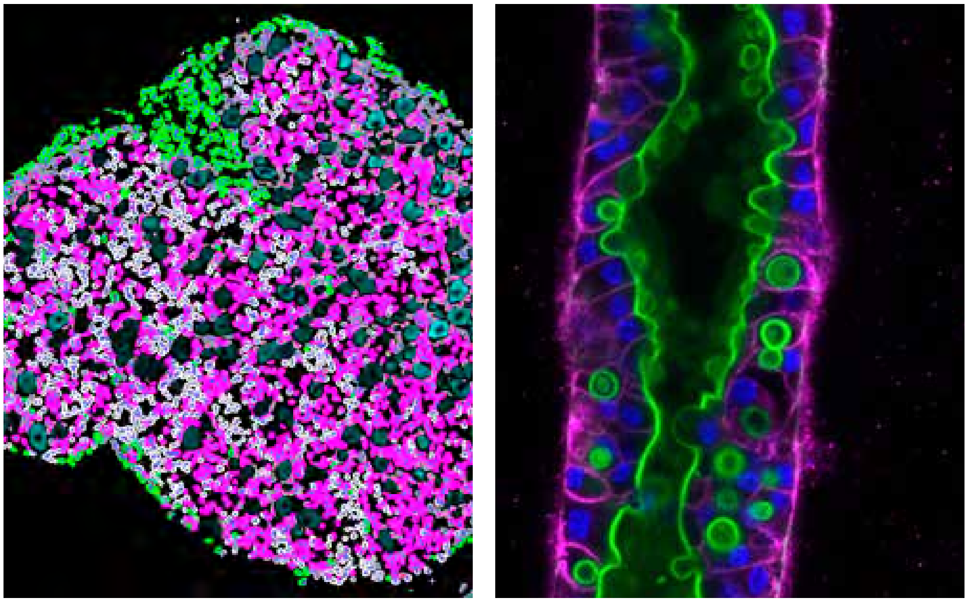
The McClatchey laboratory focuses on understanding how cells organize their outer surface – an important cellular compartment created by the interface between the cell membrane and underlying cortical cytoskeleton. This compartment governs the shape, identity and behavior of individual cells, as well as how they interact biochemically and mechanically with the extracellular environment. Normal cells modulate the features of the membrane:cytoskeleton interface to carry out key developmental processes and build functioning tissues. On the other hand, cancer cells exploit this compartment to interact inappropriately with other cells and with their environment during tumor initiation, invasion and metastasis. Our research stems from a longstanding quest to understand the molecular basis of a familial cancer syndrome caused by mutation of the *neurofibromatosis type 2 (NF2)* tumor suppressor gene. The *NF2*-encoded protein, Merlin, and closely related ERM proteins (Ezrin, Radixin and Moesin) are central architects of the cell cortex that have important roles in development and in many human cancers.

Understanding morphogenesis and tumorigenesis

The vast array of forms and functions exhibited by different cell types is enabled by the intrinsic organization of specialized domains within the cell cortex such as the leading edge of migratory cells, immunological synapse and microvillus-studded apical surfaces of epithelial cells. The spatial organization of individual cells, in turn, governs their organization into three dimensional structures that carry out organ-specific functions, such as the tubular networks of the lung, kidney, breast and liver and the heterotypic axoglial junction of peripheral nerves. The spatial organization of cortical domains in individual cells and tissues provides an essential layer of regulation to both biochemical and adhesive receptors on the cell surface. Alterations in cellular architecture are the earliest evidence of a developing tumor and signatures of tumor invasion and metastasis.

The assembly of cortical domains requires the coordination of processes occurring at the plasma membrane and underlying cytoskeleton. The overarching goal of my laboratory is to understand how the dynamic organization of this cellular compartment contributes to morphogenesis and tumorigenesis. We have focused particular attention on the neurofibromatosis type 2 (NF2) tumor suppressor, Merlin, and closely related ERM proteins (Ezrin, Radixin and Moesin) - membrane:cytoskeleton linking proteins that simultaneously influence membrane complexes and the cortical actomyosin cytoskeleton. The activities of Merlin/ERM proteins are critical for the morphogenesis of many tissues and have been implicated in many cancers.

Ongoing projects utilize mouse models, bioengineered 3D models and quantitative imaging to study the roles of Merlin/ERM proteins in the morphogenesis of intrahepatic bile ducts in the liver



Left: Digital image analysis highlights intra-tumoral heterogeneity of autocrine ligand production in a dorsal root ganglia from a six-month old Postn-Cre/Nf2flox/flox mouse. The Highplex FL algorithm in HALO imaging software was used to achieve single cell segmentation and detect neuregulin-1 positive (magenta), phospho-S6 positive (green), or neuregulin-1/phospho-S6 positive (gray) cells (in collaboration with the laboratory of Dr. Shannon Stott). *Image credit: Christine-Chiasson MacKenzie, PhD*
Right: Confocal image of a three dimensional cell culture model of biliary tube formation labelled for E-cadherin (green) and actin (magenta). *Image credit: Evan O'Loughlin, PhD*

and Schwann cell:axon relationships in peripheral nerves, and in the development of biliary and schwann cell tumors. Our studies have uncovered novel design principles that govern tissue morphogenesis and are hijacked by tumor-causing alterations, identified mechanisms by which aberrant cortical organization can drive intrinsic tumor heterogeneity, and yielded unexpected therapeutic targets and avenues of translation for cancer therapy.

It is increasingly clear that cancer fundamentally reflects the aberrant re-enactment of developmental processes. We believe that the continued partnering of discovery-based science and translational studies will lead to novel therapeutic avenues while continuing to advance our understanding of the basic cellular activities that contribute to many human cancers.

Selected Publications:

Chiasson-MacKenzie C, Vitte J, Liu CH, Wright EA, Flynn EA, Stott SL, Giovannini M and **McClatchey AI**. Cellular mechanisms of heterogeneity in NF2-mutant schwannoma. *Nat Comm* 14(1):1559, 2023 March 21.

Chiasson-MacKenzie C, Morris ZS, Liu CH, Bradford WB, Koorman T, **McClatchey AI**. Merlin/ERM proteins regulate growth factor-induced macropinocytosis and receptor recycling by organizing the plasma membrane:cytoskeleton interface. *Genes Dev*. 32(17-18): 1201-14, 2018 Sep 1.

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Hebert AM, Duboff B, Casaletto JB, Gladden, AB, **McClatchey AI**. Merlin/ERM proteins establish cortical asymmetry and centrosome position. *Genes Dev*. 26(24): 2709-23, 2012 Dec 15.

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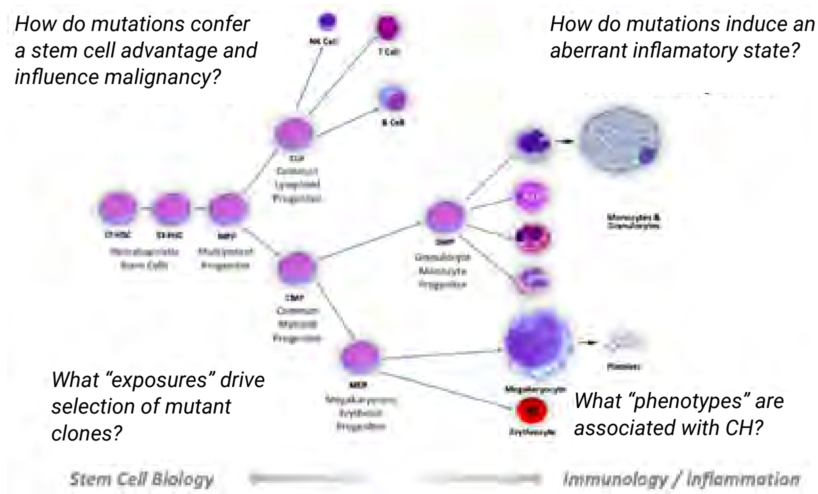
The Miller laboratory seeks to understand how somatic mutations in blood cells arise and drive abnormal cellular states including the development of blood cancers such as leukemia. We incorporate orthogonal tools including human genetics, mouse models, cellular assays, genetic screens, and molecular techniques to identify genes that are recurrently altered in blood disorders and determine how these alterations alter cellular programs such as self-renewal, response to DNA damage, and inflammation. We are particularly interested in using these tools to understand (1) the role of *PPM1D*, a gene that regulates the DNA Damage Response, in blood cell development (2) how mutations in *PPM1D* allow cells to be more resistant to chemotherapy and (3) how mutations in blood cells more generally influence inflammatory programs and pathophysiologic processes across multiple tissue-types. We seek to use our understanding of this biology to develop new therapies for the prevention and treatment of blood cancers.

Over the lifespan of an organism, somatic mutations arise in stem cells in many organs, some of which confer a competitive survival or growth advantage to the mutant cells. In such cases, a clonally selected population emerges in which additional mutational events can lead to malignant transformation and the development of cancer. This is particularly true in the blood system where mutations can drive selection of a non-malignant population, so called clonal hematopoiesis (CH), with subsequent mutational events leading to the development of blood cancers including myeloid neoplasms such as myeloproliferative neoplasms, myelodysplastic syndrome (MDS), and acute myeloid leukemia (AML). We believe that understanding the molecular mechanisms by which mutations arise in hematopoietic cells and drive neoplastic transformation can highlight novel therapeutic opportunities for the treatment of blood cancers, particularly MDS and AML.

DNA sequencing studies have informed our understanding of the genetic landscape of many hematologic malignancies, including MDS and AML. Further efforts have catalogued the genes that are mutated in CH by identifying somatic alterations present in the peripheral blood of individuals without blood cancers. Taken together, these human genetic studies can inform the timing and context in which various mutations arise, and in so doing identify critical mediators of both normal hematopoiesis and malignancy. We utilize these studies to define testable hypotheses in the lab, the results of which can further inform clinical decision-making.

Our work has largely focused on mutations in the gene *PPM1D*. Using selected patient cohorts, we have found that individuals who have received cytotoxic therapy (chemotherapy or radiation) are significantly more likely to harbor activating mutations in *PPM1D*, in the form of CH or frank malignancy (MDS or AML). We now know that these mutations, which arise in hematopoietic stem cells, lead to increased levels of PPM1D protein via impaired proteasomal degradation. This in turn allows PPM1D to suppress the DNA damage response and P53 activation more effectively, thereby allowing *PPM1D*-mutant



Framework for thinking about how somatic mutations arise in hematopoietic stem cells and drive aberrant stem cells processes including malignancy (left) and how these mutations influence aberrant inflammatory programs when present in mature immune cells and contribute to various disease phenotypes (right).

cells to have a survival advantage relative to unmutated cells in the presence of cytotoxic stress. We now seek to more deeply characterize the biological processes driving these observations using novel genetically engineered mouse models, functional genetic techniques, and biochemical assays. We hypothesize that defining the role of PPM1D in normal and malignant hematopoiesis will both drive our efforts to therapeutically target PPM1D in numerous oncologic contexts, and more broadly inform our understanding of the DNA damage response in normal and cancerous cells. This is particularly important in individuals who have therapy-related cancers that tend to be highly resistant to our standard therapies and have very poor outcomes.

We also are interested in understanding how CH mutations drive aberrant inflammatory states. Numerous groups have shown that individuals with CH have a greater risk of adverse cardiovascular outcomes, via enhanced inflammatory programs within mature, mutant immune cells. Using analogous approaches, we found that individuals with CH are more likely to have chronic obstructive pulmonary disease (COPD), particularly severe forms, and that mice with hematopoietic loss of Tet2, a gene commonly mutated in CH, have enhanced pulmonary emphysema in numerous models, akin to what is seen in human COPD. We now seek to understand which mutant blood cell types and the specific molecular pathways that drive this enhanced lung inflammation. We believe that a deep understanding of the link between CH and COPD will define new therapeutic opportunities to treat inflammatory disease of the lung and beyond.

Taken together, our lab seeks to leverage observations from human genetic studies to make clinically meaningful biological insights with the goal of developing new therapies to improve the outcome of our patients with hematologic malignancies.

Selected Publications:

Miller PG, Sperling AS, Mayerhofer C, McConkey M, Ellegast JM, Da Silva C, Cohen DN, Wang C, Sharda A, Yan N, Saha S, Schluter C, Schechter IA, Słabicki M, Sandoval B, Kahn J, Boettcher S, Gibson CJ, Scadden DT, Stegmaier K, Bhatt S, Lindsley RC, Ebert BL. PPM1D modulates hematopoietic cell fitness and response to DNA damage and is a therapeutic target in myeloid malignancy. *Blood*. 2023 Aug 18:blood.2023020331.

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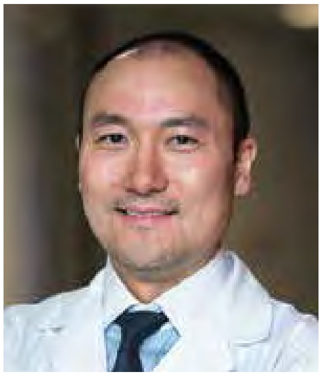
David T. Miyamoto, MD, PhD



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 analyze molecular profiles of tumor biopsies as well as circulating tumors cells (CTCs) in the blood that can be sampled non-invasively and repeatedly. By studying 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 rational selection of appropriate therapies for each individual patient with cancer.

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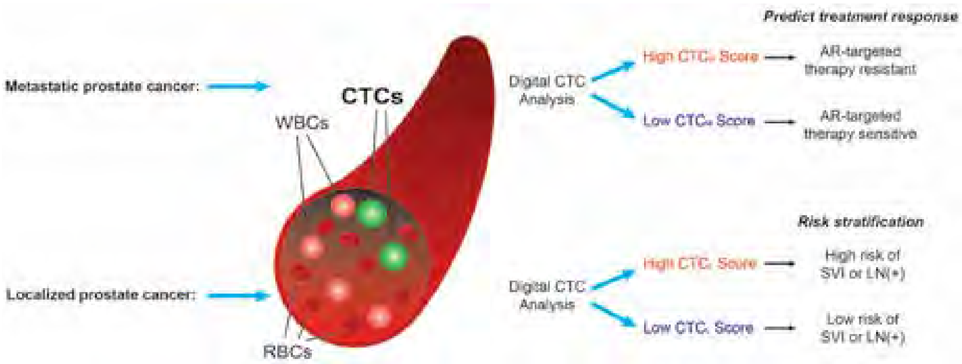
The mission of our translational research laboratory is to discover and develop molecular biomarkers that inform clinical decisions in the management of patients with genitourinary malignancies. We aim to develop circulating and tissue-based biomarkers in a variety of clinical contexts to actualize the concept "real-time precision medicine," integrating genomic analyses of liquid and tissue biopsies to guide the personalized care of patients with genitourinary malignancies.

Prostate cancer is the most common cancer in men and the second leading cause of cancer-related death in men. There is a critical unmet need for predictive biomarkers to guide the rational selection of appropriate treatment options for each patient with prostate cancer in settings ranging from localized to metastatic disease. A major focus of our laboratory is the investigation of circulating tumors cells (CTCs), which are rare cancer cells shed by primary and metastatic tumors into the peripheral blood circulation. CTCs represent a type of "liquid biopsy" that may be performed repeatedly and non-invasively to monitor treatment efficacy and study tumor evolution during therapy. As part of a collaborative, multidisciplinary team at MGH, we have developed novel molecular assays using microfluidic technologies to isolate and analyze CTCs from cancer patients. Our recent studies include the use of CTC expression profiling to interrogate signaling pathways and derive CTC RNA signatures that predict resistance to androgen receptor (AR)-targeted therapy in metastatic cancer and early dissemination in localized cancer. Ongoing projects include the development of CTC molecular signatures to predict clinical outcomes after radiation therapy as well as novel prostate cancer therapies currently in Phase 1/2 clinical trials. Another focus is the development of novel tissue-based biomarkers. We utilize technologies including next-generation sequencing and RNA in situ hybridization (RNA-ISH) to evaluate prognostic and predictive molecular signatures in limited quantities of archival prostate tumor tissues from clinical trials or carefully selected clinical cohorts. Our ongoing efforts are directed at correlating molecular findings with clinical outcomes to identify novel biomarkers predictive of treatment response that can be useful in the clinic.

Bladder cancer is the fifth most common cancer in the US, causing 18,000 deaths per year. Muscle-invasive bladder cancer has a high propensity for metastasis and requires aggressive treatment with either radical



Potential clinical applications of digital CTC analysis in metastatic and localized prostate cancer. AR, androgen receptor; CTC, circulating tumor cell; LN, lymph node; RBC, red blood cell; SVI, seminal vesicle invasion; WBC, white blood cell (Miyamoto et al. Cancer Discovery 2018).

cystectomy or bladder-sparing trimodality therapy (transurethral tumor resection followed by chemoradiation). However, the decision regarding which treatment to pursue is often made based on arbitrary factors including patient or physician preference. There is an urgent unmet need for molecular biomarkers to guide patients towards the most appropriate therapy based on the biology of their tumor. We recently performed gene expression profiling of bladder tumors from patients treated with trimodality therapy and identified immune and stromal molecular signatures predictive of outcomes after chemoradiation. Ongoing projects include the development of CTC RNA signatures to predict outcomes and monitor for minimal residual disease after bladder cancer therapy. We are currently evaluating these and other candidate biomarkers as predictors of treatment response in prospective clinical trials and carefully defined retrospective clinical cohorts.

Selected Publications:

Guo H, Vuille JA, Wittner BS, Lachtara EM, Hou Y, Lin M, Zhao T, Raman AT, Russell HC, Reeves BA, Pleskow HM, Wu CL, Gnirke A, Meissner A, Efstathiou JA, Lee RJ, Toner M, Aryee MJ, Lawrence MS, **Miyamoto DT***, Maheswaran S*, Haber DA*. DNA hypomethylation silences anti-tumor immune genes in early prostate cancer and CTCs. *Cell*. 2023; 186:2765-2782.

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Efstathiou JA, Mouw K, Gibb E, Liu Y, Wu CL, Drumm M, da Costa JB, du Plessis M, Wang NQ, Davicioni E, Feng FY, Seiler R, Black PC, Shipley WU, **Miyamoto DT**. Impact of immune and stromal infiltration on outcomes following bladder-sparing trimodality therapy for muscle-invasive bladder cancer. *European Urology*. 2019; 76:59-68.

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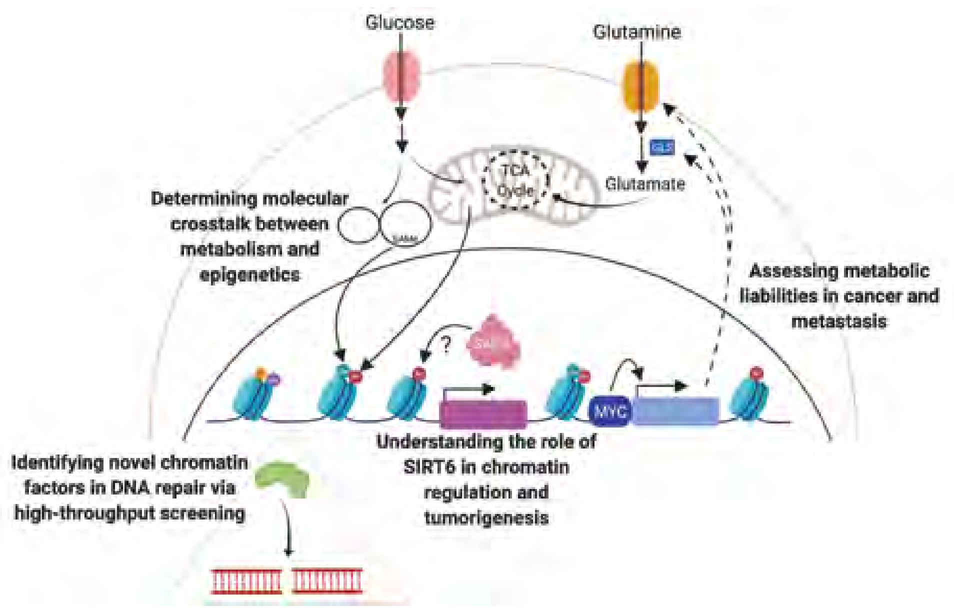
Research in **the Mostoslavsky laboratory** focuses on the crosstalk between chromatin dynamics and cellular metabolism. In particular, we have focused on sirtuins, a family of proteins first discovered in yeast that plays a critical role in many human diseases, including cancer. Most of our previous work involves the Sir2 mammalian homolog known as SIRT6, an enzyme with roles in compacting the DNA scaffolding structure known as chromatin. 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. Our current projects involve understanding the molecular roles of chromatin in DNA repair, identifying chromatin and metabolic drivers of metastatic disease, and the crosstalk between metabolic pathways and chromatin structure.

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 finely tuned 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. 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 severe hypoglycemia. SIRT6 functions as a histone H3K9 deacetylase to silence glycolytic genes; in this way directing glucose away from the TCA cycle to reduce intracellular ROS levels. This function appears critical for glucose homeostasis, as SIRT6 deficient animals die early in life from hypoglycemia. Remarkably, SIRT6 acts as a tumor suppressor in multiple cancers, regulating cancer metabolism through mechanisms that bypass 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



Understanding the crosstalk between metabolism and Epigenetics

Image Credit: Lara Roach

(i.e., the Warburg effect), the molecular mechanisms behind this metabolic switch remained a mystery. We found that SIRT6 is a critical epigenetic modulator of the Warburg effect, providing a long-sought molecular explanation to this phenomenon. Importantly, new work from the lab suggests that such metabolic adaptation occurs in a rare population of cells, indicating that tumors exhibit metabolic heterogeneity. 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. Lastly, we have also 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 in cancer cells to acquire "epigenetic plasticity".

In recent years, we have broadened our research to explore roles of one carbon metabolism (1C) in chromatin dynamics, exploring novel metabolic liabilities in cancer (uncovering a novel adaptation to bypass glutamine deprivation), new chromatin modulators of DNA repair, where we discovered a new factor that modulates Homologous Recombination, explaining some features of a human syndrome, and the use of screening strategies to identify novel epigenetic/metabolic drivers of metastatic disease. We use a number of experimental systems, including biochemical and biological approaches, as well as genetically engineered mouse models.

Specific projects:

1. Determining the role of SIRT6 in tumorigenesis using mouse models
2. Elucidating the role of histone modifications and chromatin dynamics in DNA repair
3. Determining molecular crosstalk between epigenetics and metabolism
4. Discovering non-genetic (epigenetic and metabolic) drivers of metastases

Selected Publications:

Choi-J, Sebastian C, Ferrer C, Lewis C, Sade-Feldman M, Lasalle T, Gonye A, Lopez BCG, Abdelmoula W, Regan MS, Cetinbas, M, Pascual G, Wojtkiewicz GR, Silveira GG, Boon R, Ross KN, Tirosh I, Saladi SV, Ellisen LW, Sadreyev RI, Benitah SA, Agar NYR, Hacohen N, and **Mostoslavsky R.** A unique subset of glycolytic tumor propagating cells drives squamous cell carcinoma. *Nature Metab*. 2021, 3, 182-195.

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Mo Motamedi, PhD

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Research in **the Motamedi laboratory** focuses on a molecular memory system, called epigenetics, which allows cells to develop distinct identities during development or become resistant to different types of stress such as chemotherapy. Epigenetic states are formed when groups of genes are turned on and off at a given time in a given cell. Recent work has shown that cancer cells exploit epigenetic mechanisms to develop resistance to radiation, chemo- or immune-therapy. By studying the molecular machinery that establish epigenetic states in model organisms, the Motamedi lab has identified a critical pathway that helps cancer cells establish resistance to therapy. By inhibiting this pathway, they aim to reverse chemotherapy resistance stably in several cancers. This discovery will help in addressing this difficult unmet need in cancer therapy.

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. Indeed, 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. These principles now have emerged as conserved mechanisms by which noncoding RNAs partake in chromatin regulation in eukaryotes including in humans.

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,

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.

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.

Selected Publications:

Calvo I A, Sharma S, Paulo JA, Gulka A, Boeszoermenyi A, Zhang J, Lombana JM, Palmieri CM, Laviolette LA, Arthanari H, Iliopoulos O, Gygi SP, **Motamedi M**. The fission yeast FLCN/FNIP complex augments TORC1 repression or activation in response to amino acid (AA) availability. *iScience*. 2021. 24(11), 103338.

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†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 the cover story in ***Cell***

Eugene Oh, PhD

Ubiquitylation is one of the most common protein modifications and arguably the most versatile. How this post-translational modification shapes the intracellular signaling networks that dictate specific cellular states and behaviors is a central focus of **the Oh laboratory**. We recently identified a novel ubiquitin-dependent mechanism that integrates gene expression with cellular division to preserve the identity of proliferating cell types. Our current focus is to elucidate how various cancer cell types hijack this system to confer specific proliferative and survival advantages. The goals of this exploration are to target the ubiquitin system for drug discovery and to find new strategies to rewire the gene expression landscape of cancer cells.

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How cells process information and make decisions is essential for their survival. The intracellular signaling events that ultimately evoke specific cellular responses make frequent use of ubiquitylation. Failure to properly do so can cause abnormal cell growth and uncontrolled proliferation, both hallmarks of tumorigenesis. Our lab is broadly interested in understanding the ways in which ubiquitylation gates key decision-making processes and how misregulation of this modification contributes to various malignancies.

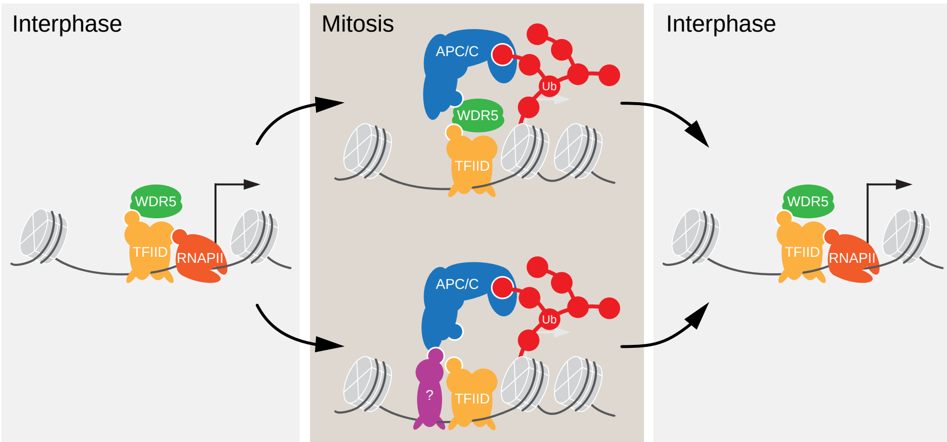
Ubiquitin-dependent control of gene expression

The identity of every cell is governed by the coordinated expression of specific gene networks. Yet dividing cells temporarily halt their transcriptional output during mitosis, thus how these cells preserve a transcriptional memory that defines their cellular state is not completely understood. Using modern genetic discovery platforms, we found that the ubiquitin ligase APC/C (anaphase-promoting complex) is required for controlling the pluripotent identity of human embryonic stem cells. Our studies revealed that the APC/C is recruited to a subset of gene promoters by the chromatin recruitment factor WDR5, which enables the APC/C to decorate nearby histone proteins with ubiquitin chains assembled through specific linkages. These ubiquitin polymers serve as potent extraction signals for the ATP-dependent segregase p97/VCP. The displacement of histone proteins removes a critical barrier to transcription, ensuring the rapid re-expression of pluripotency genes upon entry into the next cell cycle. Altogether, our work highlights an unexpected role for ubiquitylation in gene expression control.

A key implication of this mechanism is that the APC/C can direct the identity of any dividing cell type, including abnormally proliferating cancer cells. Our ongoing research focuses on identifying which cancer types are dependent on the APC/C for their identity and characterizing the molecular basis for this control. Interestingly, the APC/C binds to a number of cancer-linked transcription factors, with many of these interactions only observed in specific cancer lines, suggesting that a single enzyme can elicit a multi-faceted response by tailoring a custom gene expression program for each cancer type.

Decoding the chromatin-bound ubiquitin code

Ubiquitin can also form polymeric chains that adopt unique structures. This



A model for how APC/C controls gene activity in dividing cell types. The expression of self-renewal genes is dependent on WDR5, while the expression of cancer-specific genes requires factors that are yet to be identified.

topological diversity translates into a diversity of functional outcomes, making this modification exceptionally versatile as a regulatory system. Our lab found that the APC/C deposits defined ubiquitin polymers – linked via residues Lys11 and Lys48 – on chromatin-bound substrates. Yet whether and how other ubiquitin chain types control gene expression is unknown. Ongoing efforts in our lab include developing new strategies to probe for the various linkage types that regulate gene activity and understanding the molecular basis for these linkages. Our ultimate goal is to untangle the complexity of the chromatin-bound ubiquitin code and to decipher how this code is controlled. Major questions include understanding how specificity of this modification is achieved and whether ubiquitylation might crosstalk with other post-translational modifications.

Selected Publications:

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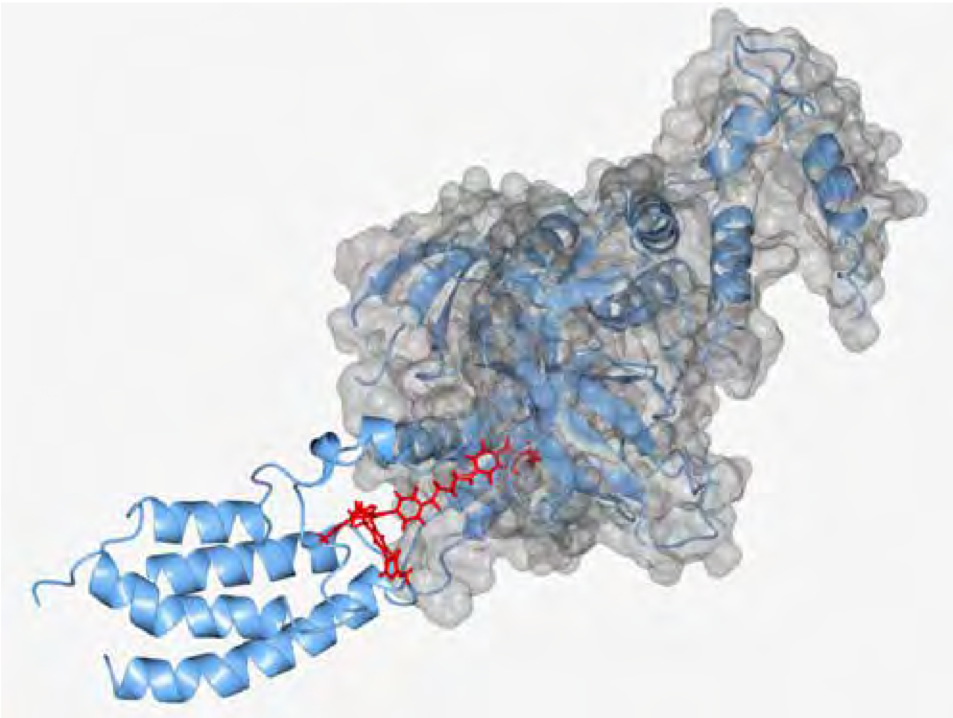
Mutations in cancer cells lead to malfunctioning control of gene expression. **The Ott laboratory** is dedicated to discovering the gene expression control factors that are essential for cancer cell survival. Discovery of these factors prompts further efforts in our group to design chemical strategies that directly target aberrant mechanisms of gene control. Biologically, gene control factors represent compelling therapeutic targets for cancer treatment as they are master regulators of cell identity. Yet despite this clear rationale, many are perceived as intractable drug targets owing to their large size, disordered shapes, and orchestration of complex cellular circuits. Recent advances in discovery chemistry, high-throughput assay technology, and gene editing technologies have advanced our capability to identify targetable components of gene control machinery. We use these chemical and genetic tools to probe cancer cells for new vulnerabilities ripe for therapeutics development.

Chemical modulation of bromodomains

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 protein modules called bromodomains. Recently, novel chemical compounds have been advanced that selectively target bromodomains. These compounds efficiently displace these proteins from enhancers, and we and others have found them to be active agents in models of acute leukemia, lymphoma, and several solid tumor types (Ott et al, *Blood* 2012; Ott et al, *Cancer Cell* 2018). Using a suite of genome-wide chromatin and transcriptomic assays, we aim to understand principles of bromodomain dependency in cancer. 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*. 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 or focal amplification. We have generated high-resolution enhancer landscapes derived from primary patient samples, including a large cohort of chronic lymphocytic leukemia samples (Ott et al, *Cancer Cell* 2018). 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



Structural model of the ternary complex formed by a novel chemical degrader of the CBP/p300 (dCBP-1) developed by the Ott laboratory. dCBP-1 (in red) induces degradation of CBP/p300 by acting as a 'molecular glue' between an E3 ubiquitin ligase and the bromodomain of CBP/p300 (structural model generated by J. Sayilgan).

for maintaining leukemia and lymphoma cell states.

Expanding the chromatin chemical probe toolbox

The successful discovery chemistry efforts that yielded bromodomain inhibitors have revealed chromatin reader domains broadly, and bromodomains specifically, as protein modules amenable for small molecule ligand development. Used experimentally, enhancer-targeting compounds enable precise and acute modulation of chromatin factors 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 elucidate fundamental aspects of epigenome 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 in cancer cells. Our recent efforts have led us to describe the first chemical degrader of the enhancer lysine acetyltransferases CBP and p300 (Vannam et al, *Cell Chemical Biology* 2021). Ongoing projects seek to expand our current toolbox of enhancer-targeting small molecules, and to develop these compounds into prototype cancer therapies.

Selected Publications:

Vannam R, Sayilgan J, Ojeda S, Karakyriakou B, Hu E, Kreuzer J, Morris R, Herrera Lopez XI, Rai S, Haas W, Lawrence M, **Ott CJ**. Targeted degradation of the enhancer lysine acetyltransferases CBP and p300. *Cell Chemical Biology*. 2021; 28:503-514.

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The focus of **the Pinello laboratory** is to use innovative computational approaches and cutting-edge experimental assays, such as CRISPR 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 AI, machine learning and high-performance computing technologies to solve computationally challenging and Big Data problems associated with functional genomics and 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.

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.

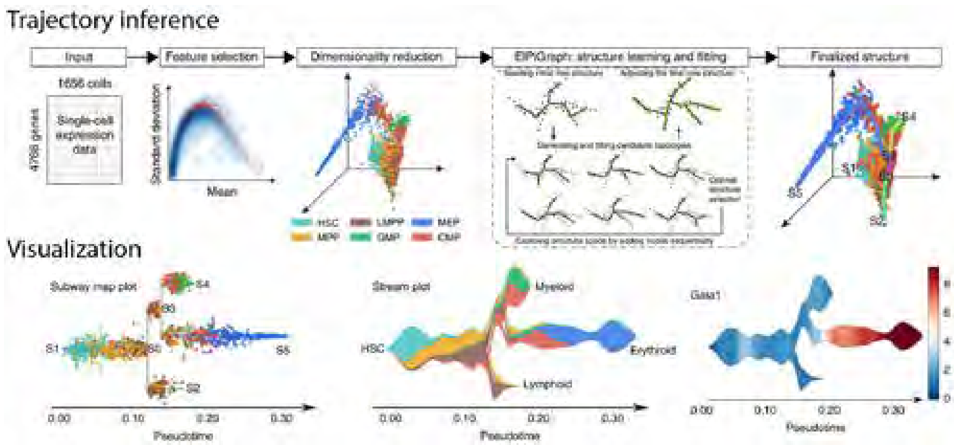
We recently developed an integrative computational pipeline called HAYSTACK HAYSTACK is a software tool (*https://github.com/lucapinello/Haystack*) to study epigenetic variability, cross-cell-type plasticity of chromatin states and transcription factor motifs and provides mechanistic insights into chromatin 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

We embraced the revolution in functional genomics made possible by the novel genome editing approaches such as CRISPR/Cas9, base editing and prime editing by developing computational tools for the design, quantification of CRISPR edits and for the analysis of coding and non-coding tiling screens for functional genomics.

We have developed CRISPResso2 (*http://crispresso2.pinellolab.org*), a software for the quantification of genome editing events that is now the standard de facto for the genome editing community. In collaboration with the groups of Daniel Bauer and Stuart Orkin, we applied our computational strategies to aid the development of several CRISPR screens for dissecting enhancer functionality in the blood system.

We have also recently developed a powerful tool called CRISPRme, which considers both SNPs and indel genetic variants to identify and prioritize off-target sites, offering a more comprehensive and accurate assessment of off-target risks. By utilizing CRISPRme, we discovered and validated a previously overlooked off-target site for a guide RNA (gRNA) targeting the BCL11A enhancer, currently being used in clinical trials for sickle cell disease and β-thalassemia.



STREAM on transcriptomic data from the mouse hematopoietic system. Top, STREAM workflow to recover hierarchical structure composed of curves approximating the inferred trajectories. Single cells are represented as circles and colored according to the FACS sorting labels. Bottom, from left to right, Subway map plot representation at single cell resolution; branches are represented as straight lines. The length of the branches and the distances between cells and assigned branches are proportional to the original representation in the 3D space. Rainbow plot: intuitive visualization to show cell type distribution and density along different branches. Relative expression of GATA1 in each branch using the reconstructed structure.

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 developing tools to characterize cellular types and states at single cell resolution by using data from single cell transcriptomic or epigenomics data. For example, we developed STREAM (Single-cell Trajectories Reconstruction, Exploration And Mapping), an interactive computational pipeline for reconstructing complex cellular developmental trajectories from sc-qPCR, scRNA-seq or scATAC-seq data available at *http://stream.pinellolab.org*. This method can be used for disentangling complex cellular types and states in development, cancer, differentiation or in perturbation studies.

Selected Publications:

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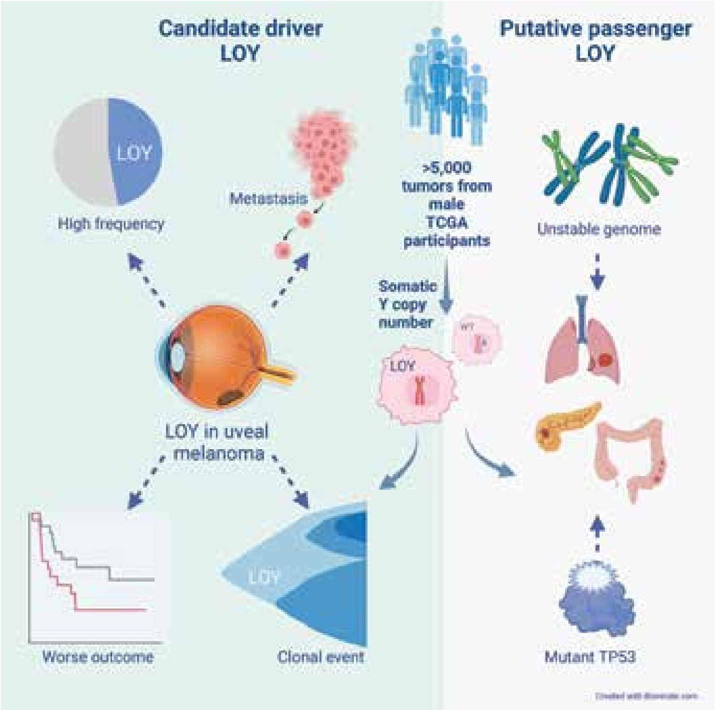
Most known genomic drivers of cancer are in coding genes, affecting the encoded protein's interaction with other proteins, DNA or biological compounds. Recent advances in DNA sequencing technology have made it possible to study non-coding regions that regulate these protein-coding genes. Several cancer 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 the contribution of the sex chromosomes, especially the Y chromosome, to cancer. Loss of Y is known to be associated with morbidity and mortality in aging men, yet its role in tumors is largely unclear. Much of this is due to technical challenges that our group aims to solve. Understanding the driver genes on the sex chromosomes will help us explain differences in male and female tumors, and forge a path to more effective, sex-informed treatment.

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.

Role of the sex chromosomes in cancer

Cancer affects men and women disparately, with strong differences in incidence and



Y chromosome loss in cancer can be a driver (left) or passenger (right) event.

outcome in some tumor types. Human sex is determined by the sex chromosomes X and Y. Because men only have one X chromosome, they are particularly vulnerable to congenital and acquired somatic variants in X-linked genes. It has been shown that both sex chromosomes can be lost in both normal blood cells with age, as well as certain tumor cells. Yet the meaning of Y chromosome loss, and possible cancer genes on this chromosome, are poorly understood. This is because Y is technically challenging to study with commonly used 'omics' profiling approaches. We develop analysis strategies and methods to tackle these technical challenges and use them to find new X and Y-linked drivers in published tumor genome sequences. Our goal is to identify sex-specific, and potentially targetable, vulnerabilities in human cancer.

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Research in **the Rivera laboratory** focuses on using genomic tools to identify and characterize gene regulation pathways that are altered in cancer. An important feature shared by most tumors is the dysregulation of complex gene expression programs 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 mechanisms and pathways. Given that the mechanisms that drive changes in gene expression programs in cancer are poorly understood, we anticipate that our studies will point to new therapeutic approaches.

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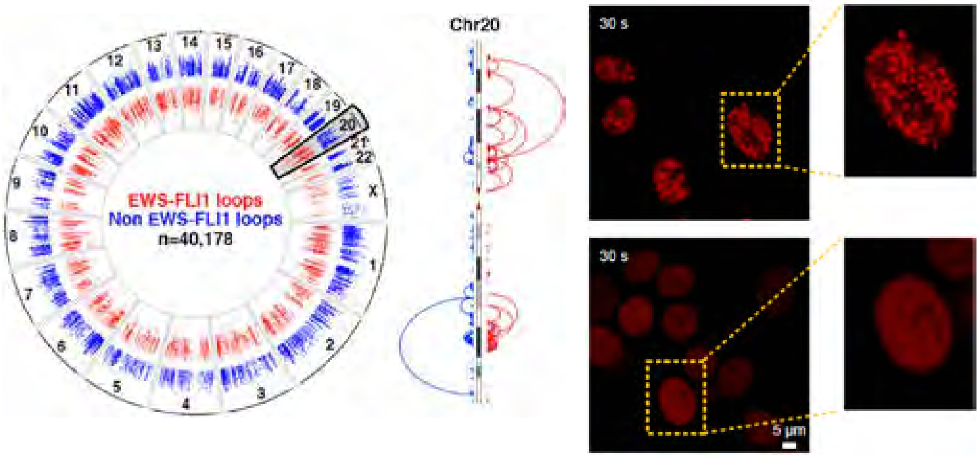
Epigenomic approaches for the identification of novel pathways in cancer

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, 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 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. Recently, our work has also incorporated new 3D chromatin configuration technologies (e.g. HiC and HiChiP) that can measure the critical contributions of spatial organization to gene regulation in a genome-wide scale.

We have performed extensive chromatin profiling of several tumor types, including pediatric tumors such as Ewing sarcoma and medulloblastoma 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



Looping patterns and IDR condensation in Ewing sarcoma cells. Left panels: The oncogenic transcription factor EWS-FLI1 is a dominant force in establishing the 3D configuration of DNA in Ewing sarcoma. EWS-FLI1 accounts for almost half of all loops in tumor cells (shown as red dots in the circle plot and as loops for a magnified view of Chromosome 20. Right panels: Optogenetic experiment showing induction of condensates by a transcription factor with an intrinsically disordered domain (IDR, top). This effect is lost if the IDR is removed (bottom).

poorly defined. These analyses have led us to identify new therapeutic targets for tumors where no targeted therapies are currently available.

Role of intrinsically disordered regions (IDRs) in cancer

Our studies of gene regulation in cancer have led us to identify unexpected oncogenic mechanisms that have broad implications. In particular, our work has shown that the intrinsically disordered region (IDR) of the EWS-FLI1 oncogenic fusion protein is essential for its function and enables the activation of tumor specific regulatory elements. Given that EWS-FLI1 is part of a large group of fusion oncogenes that share the same disordered domains, we have used this insight to study similar mechanisms in other tumor types (e.g. Clear Cell Sarcoma). Moreover, IDRs are present in many other oncogenes involved in gene regulation and we are developing new methods to study these domains. For example. we recently developed DisP-seq, a method that allows us to identify genomic locations with high concentrations of IDRs. Similarly, given that IDRs often form condensates that can promote gene activation, we are using optogenic tools to study IDRs from different transcription factors involved in cancer.

Selected Publications:

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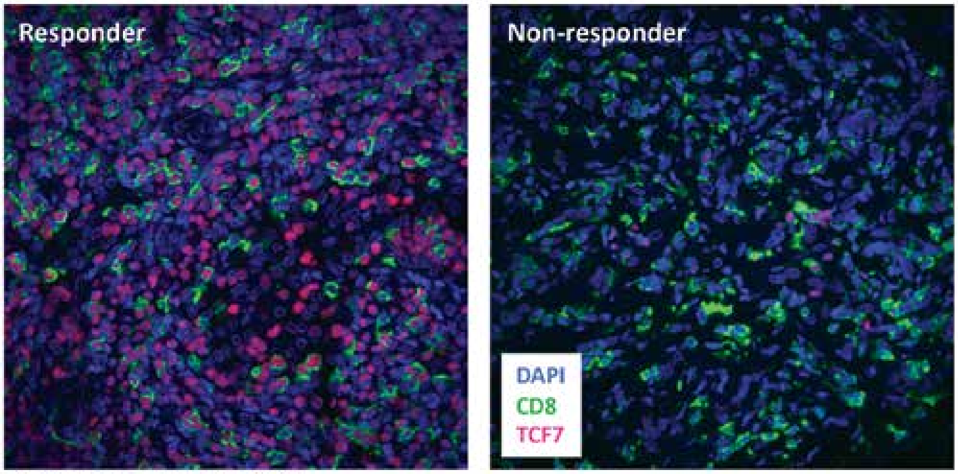
The Sade-Feldman laboratory focuses on identifying response and resistance mechanisms in cancer patients treated with immunotherapies. In the last decade, the treatment of solid tumors has been revolutionized by the development and FDA approval of checkpoint blockade (CPB) immunotherapies. While long-lasting responses are induced, only a small subset of patients benefits from these treatments. Thus, identifying the key components that drive or prevent effective immunity against tumors remains an unmet clinical need. Treatment response to immunotherapy and other therapies (e.g., targeted and chemotherapies) is influenced by complex interactions between multiple cell types in the tumor microenvironment (TME) and the heterogeneous population of tumor cells. The Sade-Feldman laboratory integrates single-cell multi-omics methods, computational biology, patient data-driven functional genomic screens, and detailed mechanistic studies to delve deeper into this intricate ecosystem and the mechanisms behind therapy response and resistance. Combining these approaches enables us to understand resistance mechanisms to immunotherapy, predict patient response, prioritize targets for validation, and identify new drug targets and combinations for cancer treatment.

While there have been numerous successful trials and FDA approvals of antibodies that block the immune regulatory checkpoints, CTLA4, PD-1, PD-L1, and LAG3, for the treatment of multiple cancer types, most patients will not respond and will succumb to the disease. The success of these immune-based therapies mainly relies on identifying tumor antigens presented on MHC-I molecules by cytotoxic immune cells. Working together with scientists, computational biologists, oncologists, surgeons, and pathologists at Mass General, our lab has discovered several mechanisms underlying the control of tumors by the immune system: I. Point mutations, deletions, or loss of heterozygosity (LOH) in beta-2-microglobulin (B2M) as a resistance mechanism to immunotherapy (Sade-Feldman et al. *Nature Comm* 2017); II. High expression of the transcription factor TBX3 in de-differentiated malignant cells as a resistance mechanism (Freeman et al. *Cell Reports Med* 2022); III. T cell states associated with clinical outcomes in melanoma patients treated with CPB inhibitors (Sade-Feldman et al. *Cell* 2018); IV. Inflammatory factors that control the differentiation and function of suppressive myeloid cells (MDSCs) (Sade-Feldman et al. *Immunity* 2014) and their clinical significance in melanoma patients treated with CPB inhibitors (Sade-Feldman et al. *Clinical Cancer Research* 2015); and V. Interferon-induced APOBEC3 as an acquired resistance mechanism to CPB in HNSCC (Lin et al. *NPJ Precis Oncol* 2022) and the prognostic impact of CXCL9/SPP1 polarity of tumor-associated macrophages in HNSCC patients with recurrent advanced disease (Bill R et al. *Science* 2023).

While these studies enabled us to understand some mechanisms of resistance to checkpoint blockade immunotherapy, still many questions remain open:

1. Despite the FDA approval of standard chemotherapy with immune checkpoint blockade (in NSCLC, SCLC, and HNSCC),



Ref: Sade-Feldman et al. *Cell* 2018

Representative overlaid images of melanoma tumors from responder and non-responder patients stained with DAPI (blue), CD8 (green), and TCF7 (red). A higher proportion of CD8+TCF7+ at baseline is observed in patients who responded to anti-PD1 immunotherapy.

we still don't fully understand how drug A affects the activity of drug B and the contribution of each drug to therapy resistance when combined.

2. Are there any shared primary or acquired resistance mechanisms between different diseases (e.g., melanoma, NSCLC, and HNSCC)?
3. While our translational efforts generate many hypotheses and predictors of outcomes, we still don't know the function of those genes/pathways and their impact on treatment response.
4. Can we identify ways to overcome resistance mediated by the loss of antigen presentation by perturbing tumor intrinsic pathways?
5. To date, most of our efforts have been focused on patients with metastatic disease receiving immunotherapy. However, there is an unmet clinical need to identify targets that can synergize with traditional therapies for local and recurrent advanced disease, particularly in cancers with a poor response to such treatments.

To address the above questions, we use a systems biology approach that involves three main steps: I. discover cellular and molecular factors associated with effective/failed therapy using integrative analysis of single-cell multi-omics datasets from human tumors; II. Perform systematic functional genetic screens to determine the role of human genes associated with outcomes; III. Characterize the key sensitivity/resistance mechanisms to understand the intra- and inter-cellular circuits underlying their action.

Main current projects in the lab:

1. Identify and validate factors conferring sensitivity and resistance to patients treated with mono or combinatorial (e.g., targeted and chemotherapy) immunotherapy by bridging together analyses of human tumors with systemic perturbations and mechanistic studies in animal and human models.
2. Identify tumor intrinsic pathways that can sensitize cells to immunotherapy in the absence of the MHC-I antigen-presentation machinery.
3. Discover targets to overcome radiation and chemotherapy resistance in local and recurrent advanced cancers.

By combining detailed human observations and rigorous functional tests, these studies are expected to reveal the basis for therapeutic resistance and response, creating a roadmap for identifying targets for therapeutic development.

Selected Publications:

Bill R, Wirapati P, Messemaker M, Roh W, ..., Lin D, Pai SI, **Sade-Feldman M**, Pittet MJ. CXCL9:SPP1 macrophage polarity identifies a network of cellular programs that control human cancers. *Science*. 2023 Aug 4;381(6657):515-524.

LaSalle TJ, Gonye ALK, Freeman SS, Kaplonek P, ..., Filbin MR, Villani AC, Hacohen N, **Sade-Feldman M**. Longitudinal characterization of circulating neutrophils uncovers phenotypes associated with severity in hospitalized COVID-19 patients. *Cell Rep Med*. 2022 Sep 26:100779.

Freeman SS*, **Sade-Feldman M***, Kim J, Stewart C, ..., Stemmer-Rachamimov AO, Wargo JA, Flaherty KT, Sullivan RJ, Boland GM, Meyerson M, Getz G, Hacohen N. Combined tumor and immune signals from genomes or transcriptomes predict outcomes of checkpoint inhibition in melanoma. *Cell Rep Med*. 2022 Feb 15;3(2):100500.

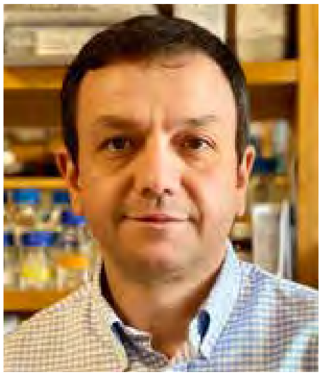
Sade-Feldman M*, Yizhak K*, Bjorgaard SL, Ray JP, de Boer CG, Jenkins RW, ..., Barbie DA, Stemmer-Rachamimov A, Flaherty KT, Wargo JA, Boland GM, Sullivan RJ, Getz G, Hacohen N. Defining T Cell States Associated with Response to Checkpoint Immunotherapy in Melanoma. *Cell*. 2019 Jan 10;176(1-2):404.

Sade-Feldman M*, Jiao YJ*, Chen JH, Rooney MS, ..., Corcoran RB, Lawrence DP, Stemmer-Rachamimov A, Boland GM, Landau DA, Flaherty KT, Sullivan RJ, Hacohen N. Resistance to checkpoint blockade therapy through inactivation of antigen presentation. *Nat Commun.* 2017 Oct 26;8(1):1136.

Sade-Feldman M*, Kanterman J*, Klieger Y, Ish-Shalom E, Olga M, Saragovi A, Shtainberg H, Lotem M, Baniyash M. Clinical Significance of Circulating CD33+CD11b+HLA-DR- Myeloid Cells in Patients with Stage IV Melanoma Treated with Ipilimumab. *Clin Cancer Res*. 2016 Dec 1;22(23):5661-5672.

* Co-first authorship

Ioannis Sanidas, PhD



Sanidas Laboratory

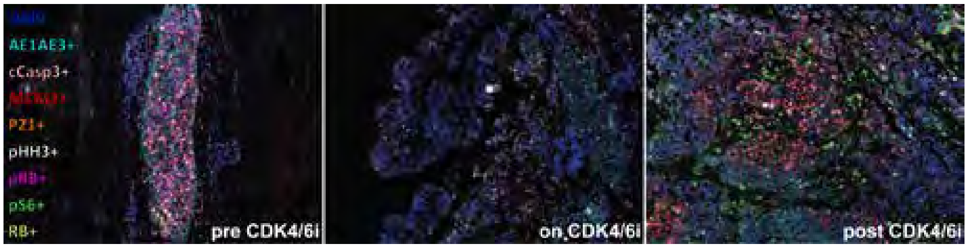
Ioanna Gkotinakou, PhD
Izabela Panova
Ioannis Sanidas, PhD
Alice Zheng

Cell cycle deregulation is a hallmark of cancer. **The Sanidas laboratory** examines the cell cycle in normal and cancer cells to discover vulnerabilities that can lead to novel therapeutic approaches. Our research primarily centers on the retinoblastoma tumor suppressor protein (RB), a key regulator of the cell cycle. RB is highly expressed in normal and cancer cells and prevents cells from dividing. Cyclin-dependent kinases (CDKs) phosphorylate and inactivate RB to enable cell proliferation. However, this description explains only a part of RB's activity; many additional functions have been attributed to RB, which are context-specific and mostly uncoupled from cell cycle regulation. This is part of the reason that although RB is genetically or functionally inactivated in most human cancers, its tumor suppressor activity is highly tissue type-specific. Understanding the molecular complexity of RB will allow us to identify the context-specific implications of its inactivation in human malignancies and optimize the advantages of the recently developed CDK inhibitors, which target the pharmacological activation of RB.

Over the last decade, a substantial amount of research has been devoted to molecular therapeutics targeting RB's activation, leading to the development of highly selective CDK inhibitors. These efforts have resulted in advanced cancer therapy methods, significantly prolonging the survival rate in Breast Cancer (BrCa) patients. Despite the widespread deregulation of the RB pathway in cancer cells, the effectiveness of these drugs remains limited to specific tumor types. At the Sanidas laboratory, we aim to address this conundrum through two lines of investigation: 1) Understanding the molecular complexity of RB and deciphering the context-specific implications of RB inactivation in cancer cells. 2) Investigating how CDK inhibitors work in various tumor types, with the goal of enhancing drug efficacy and determining the group of patients that will primarily benefit from this treatment.

Investigation of RB's mechanism of action

RB has often been described as a highly conserved cell cycle regulator with a universal mechanism of action. According to this conventional model, RB targets the E2F promoters to suppress the expression of cell cycle genes. This interaction is dependent on the cell cycle and inhibited by CDKs. However, this description explains only a part of RB's activity. RB is essential for the control of multiple transcriptional programs, the maintenance of chromosome stability, the commitment to cell lineage, and the emergence of drug resistance in cancer cells. These RB functions are context specific and largely independent of RB/E2F regulation. It is acknowledged that additional investigations are required to decipher the mechanisms governing this "non-canonical" RB activity. A significant obstacle hindering progress in this area has been that the RB research community has never really figured out how to deal with the molecular complexity of RB. Many studies have focused on the consequences of RB loss without being able to capture the details of RB in action. In the Sanidas laboratory, we have successfully developed sophisticated molecular tools to unravel the complexity of



The expression of the cell cycle marker phosphorylated RB (in pink) and the DNA replication marker MCM2 (in red) showed significant inhibition of active cell proliferation during treatment with the CDK4/6 inhibitor. However, upon developing resistance to CDK4/6 inhibition therapy, both markers were observed to be re-expressed. Multiplex imaging on human ER-positive Breast Cancer tumor biopsies pre-, on-, and post-treatment with CDK4/6 inhibitor. Tumor sections were stained for cytokeratins AE1/AE3 (epithelial cells marker), cleaved Caspase-3 (cCasp3), DAPI (DNA), MCM2, p21, phospho-Histone 3 Ser10 (pHH3), phospho-RB Ser807/Ser811 (pRB), phospho-S6 (pS6), total RB (RB), and DAPI, each represented by distinct colors.

RB's action. Precisely, we can now dissect RB into its distinct functional forms (Sanidas et al., 2019), separate the different pools of the chromatin-associated RB (Sanidas et al., 2022), and identify, using Micro-C analysis, the RB-mediated regulation of chromatin organization. These groundbreaking tools can finally provide the information needed to study RB. We aim to i) define the cell type-specific functions of RB, ii) elucidate why RB's tumor suppressor activity varies among different tumor types, and iii) determine the factors contributing to the tumor type-specific efficacy of drugs targeting RB activation. With the aid of these innovative tools, we can look into RB's mechanism of action with a significantly improved resolution, shedding light on previously uncharted aspects of RB's activity in cancer biology.

Targeting the cell cycle machinery in cancer therapy

The activation of RB's tumor suppressor activity represents a pivotal approach in molecular cancer therapeutics. Current strategies for recurrent, adjuvant, and de novo metastatic therapy in Estrogen Receptor-positive BrCa involve CDK4/6 inhibitors combined with hormonal therapy. Phase I clinical trials are underway for CDK2-specific inhibitors, targeting Cyclin E-amplified tumors, as well as tumors that progressed after CDK4/6 inhibition therapy. The Sanidas laboratory collaborates with the Termeer Center for Investigational Cancer Therapeutics at Mass General to study the mechanism of action of novel investigational drugs that target the cell cycle machinery. The efficacy of these drugs relies on the tumor type, genetic background, and treatment history. Our goals are to: i) optimize the cell cycle drugs' efficacy by defining their synergistic activity with other agents, and ii) identify biomarkers that predict response to these drugs.

Selected Publications:

Krishnan B, **Sanidas I***, Dyson NJ*. Seeing is believing: the impact of RB on nuclear organization. *Cell Cycle*, 2023 May 3;1-10.

Sanidas I, Lee H, Rumde PH, Boulay G, Morris R, Golczer G, Stanzione M, Hajizadeh S, Zhong J, Ryan MB, Corcoran RB, Drapkin BJ, Rivera MN, Dyson NJ, Lawrence MS. Chromatin-bound RB targets promoters, enhancers, and CTCF-bound loci and is redistributed by cell-cycle progression. *Mol Cell.* 2022 Aug 12:S1097-2765(22)00710-9.

Witkiewicz AK, Kumarasamy V, **Sanidas I**, Knudsen ES. Cancer cell cycle dystopia: heterogeneity, plasticity, and therapy. *Trends Cancer*. 2022 May 19:S2405-8033(22)00093-0.

Krishnan B, Yasuhara T, Rumde P, Stanzione M, Lu C, Lee H, Lawrence MS, Zou L, Nieman LT, **Sanidas I***, Dyson NJ*. Active RB causes visible changes in nuclear organization. *J Cell Biol*. 2022 Mar 7;221(3):e202102144.

Sanidas I, Morris R, Fella KA, Boukhali M, Tai EC, Ting DT, Lawrence MS, Hass W and Dyson NJ. A code of mono-phosphorylation modulates the function of RB. *Mol Cell* 2019, Mar 7;73(5):985-1000.

* Denotes equal contribution

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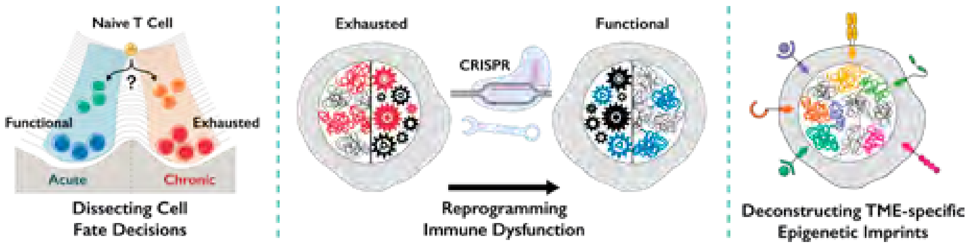
Dysfunction of the immune system is central to disease progression in cancer. **The Sen laboratory** investigates the regulation of T cell dysfunction in tumors and explores epigenetic approaches for T cell engineering. Our work lies at the interface of human immunology, systems biology, and functional epigenomics – merging clinical observations with mechanistic mouse studies to develop novel therapeutic strategies. We have found that the regulatory "circuitry" of dysfunctional T cells differs remarkably from functional T cells fighting off acute viruses. By comparing chronic viral infections and cancer, we demonstrate that this altered epigenetic wiring is a fundamental adaptation to chronic diseases and cannot be rescued by current treatments. Therefore, improved understanding of this altered regulation will be critically important for reversing cancer-associated immune dysfunction. We also pinpoint a radical new approach where we can "tune" specific components of the circuitry in immune cells to remedy their pathological state in cancer while preserving their physiological role in other contexts, thereby minimizing unwanted side-effects in patients.

Effective immunotherapy responses have been limited in 50-70% of patients, in part due to the development of T cell exhaustion wherein CD8+ T cells become dysfunctional and fail to control tumor growth. Despite ongoing clinical efforts to target exhaustion, the fundamental mechanisms specifying this state, and the potential for reinvigorating exhausted T cells, remain poorly understood.

Cell fate and behavior are governed at the level of the epigenome, through transcription factors (TFs) binding to regulatory enhancers. Therefore, we have used the gold-standard mouse model of chronic viral infection to ask whether distinct epigenetic regulation drives CD8+ T cell exhaustion. To overcome technical limitations imposed by low cell numbers, we performed ATAC-seq in exhausted cells and profiled the landscape of accessible chromatin, which is enriched for active enhancers and other regulatory elements. These studies revealed for the first time that exhausted cells acquire an extensive, state-specific epigenetic program that is distinct from memory T cells. We then integrated systems-level characterization of T cell state with CRISPR/Cas9-based enhancer editing in mouse T cell lines to show that these putative enhancers are organized into functional modules and can directly regulate exhaustion-associated genes such as PD-1.

We have sought to translate these findings to other disease contexts. First, by comparison of mouse T cells to those isolated from HCV and HIV chronic infection, we identified a conserved epigenetic program of exhaustion across species. Second, using a mouse melanoma model, we found that tumor-specific CD8+ T cells also share critical epigenetic and transcriptional features with chronic viral infection. Thus, we address a long-standing controversy about how T cell states in cancer relates to chronic viral infection by showing that T cell exhaustion is a fundamental immune adaptation to settings of chronic stimulation. Simultaneously, we have identified epigenetic signatures unique to either disease paradigm, highlighting our ability to define context-specific regulation in an unbiased way.



Leveraging the epigenetic regulation of T cell exhaustion to address fundamental and translational questions: How do T cells commit to exhaustion? How can we rescue exhausted T cells? How do disease-specific tumor microenvironments (TME) shape T cell exhaustion?

Nevertheless, major questions still remain about whether the exhausted epigenetic state is fixed or plastic in response to current treatment modalities. Recently, we examined two of the most prominent therapies to treat chronic infection and cancer: curative anti-viral regimens and immune checkpoint blockade, respectively. In chronic infection, ATAC-seq analysis of HCV-specific CD8+ T cells after cure of viremia did not reverse canonical features of exhaustion, including active super-enhancers near key TFs. In cancer, anti-PD-1 treatment of melanoma tumors also could not rescue the exhausted epigenetic state. T cell exhaustion is therefore an evolutionarily conserved epigenetic state that becomes fixed and is not reversed by some of the most common therapies.

It is becoming evident that alleviating T cell exhaustion will require new targeted approaches to reprogram exhausted cells. Our studies strongly suggest that large-scale epigenetic analysis, paired with precise CRISPR/Cas9 manipulation, will provide a roadmap for rational engineering to prevent T cell exhaustion and improve patient outcomes. To accomplish this, my lab focuses on the following:

1. Dissecting epigenetic mechanisms that govern early differentiation of CD8+ Tcells *in vivo*
2. Defining context-dependent epigenetic map of T cell dysfunction to guide patient therapies
3. Engineering exhaustion-resistant CD8+ T cells through epigenetic manipulation

These projects will generate new insights into the mechanisms and contexts in which T cell exhaustion develops in order to better design patient-specific immunotherapy regimens. In addition, they will enable unprecedented context-specific manipulation of T cell responses and create an integrative framework for characterizing and reprogramming epigenetic regulation of immune dysfunction.

Selected Publications:

Weiss SA, Huang A, Fung ME, Chen C,... Doench JG, Haining WN, Sharpe AH*, **Sen DR**.* Deletion of a state-specific PD-1 enhancer modulates exhausted T cell fate and function. *Nature Immunology* (in revision)

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Paper was highlighted on the cover of the Aug 2021 issue of Nature Immunology.

Collier JL*, Weiss SA*, Pauken KE, **Sen DR**, Sharpe AH. Not-so-opposite ends of the spectrum: CD8+ T cell dysfunction across chronic infection, cancer, and autoimmunity. *Nature Immunology*. 2021 Jul;22(7):809-819.

Brown FD, **Sen DR**, Godec J, LaFleur MW,...Sharpe AH, Haining WN, Turley SJ. Fibroblastic reticular cells enhance T cell metabolism and survival via epigenetic remodeling. *Nature Immunology.* 2019 Oct 21.

Miller BC*, **Sen DR***, Al-Abosy R, Bi K,...Hodi FS, Rodig SJ, Sharpe AH, Haining WN. Subsets of exhausted CD8+ T cells differentially mediate tumor control and respond to check-point blockade. *Nature Immunology*. 2019 Mar;20(3):326-336.

Sen DR*, Kaminski J*, Barnitz RA, Kurachi M,...Chung RT, Allen TM, Frahm N, Lauer GM, Wherry EJ, Yosef N, Haining WN. The epigenetic landscape of T cell exhaustion. *Science*. 2016 Dec 2;354(6316): 1165-1169.

Paper was highlighted on the cover of the Dec 2016 issue of Science.

*Equal contribution

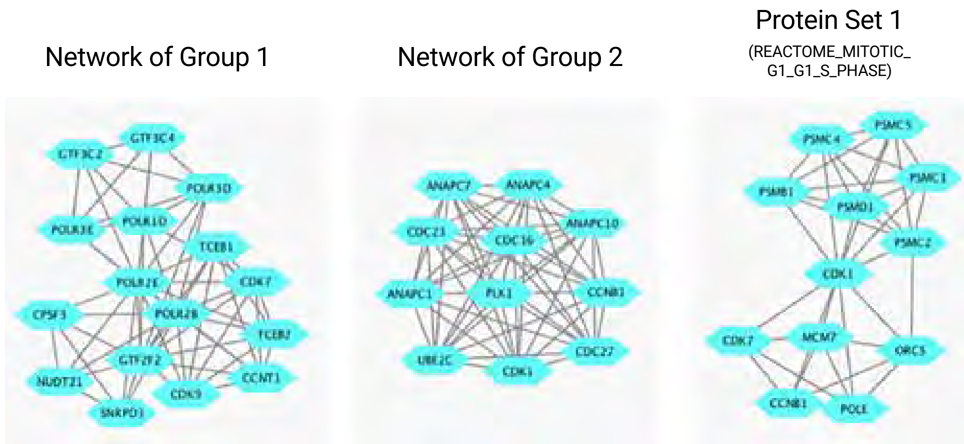
Dennis Sgroi, MD

Sgroi Laboratory

Dennis Sgroi, MD
Marinko Sremac, PhD

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 various high-throughput genetic and proteomic 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.

Presently, my laboratory is focused on applying high-throughput molecular technologies to identify biomarkers that will predict the clinical behavior of human estrogen receptor positive breast cancer in the setting of specific hormonal and chemotherapeutic regimens. We have developed the Breast Cancer Index (BCI) biomarker which is an algorithmic gene expression–based signature comprised of two functional biomarker panels, the Molecular Grade Index (MGI) and the two-gene ratio, HOXB13/IL17BR (H/I), that evaluate tumour proliferation and estrogen signalling, respectively. Integration of MGI and H/I generates a prognostic BCI score quantifying the risk of overall (0-10 years) and late (5-10 years) distant recurrence in ER+ HER2- breast cancer patients. The predictive component of BCI, the H/I ratio (henceforth BCI-H/I), has been shown to significantly predict endocrine response across several different treatment scenarios. In ER+ HER2- breast cancer patients in the extended endocrine setting, BCI predicted benefit from an additional 5 years of letrozole after ~5 years of initial tamoxifen in the MA.17 study, and most recently BCI predicted benefit from an additional 5 years of tamoxifen after 5 years of initial tamoxifen in the aTTom trial. These data provided further validation and established BCI as a unique biomarker that can help inform the decision to extend or not extend endocrine therapy beyond 5 years. BCI has been adopted in the most recent 2022 ASCO and NCCN guidelines. We are currently collaborating with the NSABP to assess



STRING network of leading edge dysregulated groups of proteins for HOXB13/IL17BR-low ER+ breast cancers. Several intriguing clusters of dysregulated protein-protein interactions are associated with the regulation of CDK4/6, cyclin E1, cyclin B1 and CDK1.

our biomarker in the NSABP-42. Lastly, we are currently studying protein-protein dysregulations in H/I-low breast cancers to identify therapeutic vulnerabilities. In a comparative analysis of H/I-high versus H/I-low breast cancers, we have identified several dysregulated pathways that may be susceptible to therapeutic intervention.

Selected Publications:

Sgroi DC, Treuner K, Zhang, Y, Piper T, Salunga R, Ahmed I, Doos L, Thornber S, Taylor KJ, Brachtel E, Pirrie S, Schnabel CA, Rea D, Bartlett JMS. Correlative studies of the Breast Cancer Index (HOXB13/IL17BR and ER, PR, AR, AR/ER ratio and Ki67 for prediction of extended endocrine therapy benefit: a Trans-aTTo study. *Breast Cancer Res*. 2022 Dec 16;24(1):90.

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Jerevall PL, Brock J, Palazzo J, Wieczorek T, Misialek M, Guidi AJ, Wu Y, Erlander MG, Zhang Y, SchnabelCA, Goss PE, Horick N, **Sgroi DC**. Discrepancy in risk assessment of hormone receptor positive early-stage breast cancer patients using breast cancer index and recurrence score. *Breast Cancer Res Treat*. 2019 Jan;173(2):375-383.

Sgroi DC, Sestak I, Cuzick J, Zhang Y, Schnabel CA, Schroeder B, Erlander MG, Dunbier A, Sidhu K, Lopez-Knowles E, Goss PE, and Dowsett M. Prediction of late distant recurrence in patients with oestrogen-receptor-positive breast cancer: a prospective comparison of the Breast Cancer Index (BCI) assay, 21-gene recurrence score, and IHC4 in TransATAC study population. *Lancet Oncol*. 2013 Oct;14(11):1067-76.

*Denotes equal contribution

Toshihiro Shioda, MD, PhD

Shioda Laboratory

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Junko Odajima, PhD
Keiko Shioda, RN, BS
Toshihiro Shioda, MD, PhD

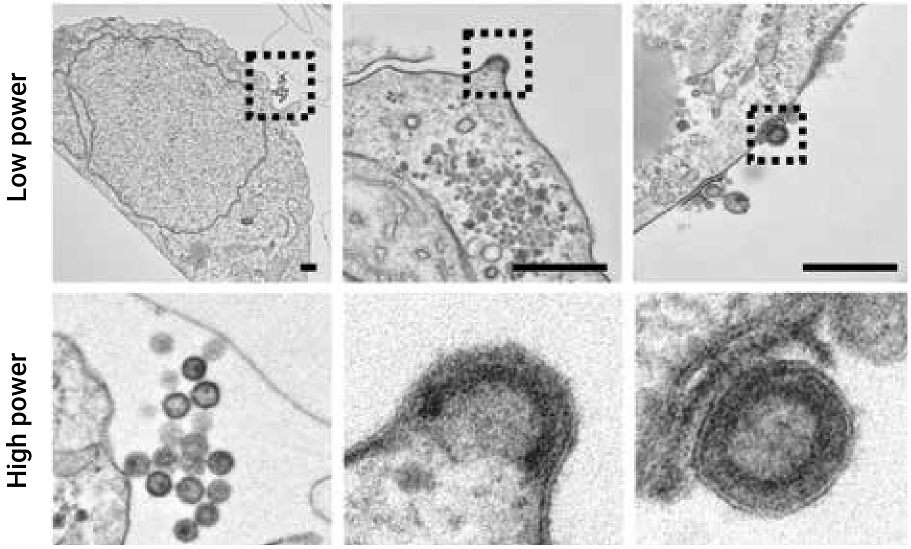
The Shioda laboratory is interested in Primordial Germ Cells (PGCs), the common precursor of gametes. Since access to PGCs in human embryos is limited, iPS cell-derived PGC-Like Cells (PGCLCs) play important roles in studying PGCs, but their lifespan is very short. Our breakthrough Long Term Culture (LTC) protocol supports perpetual expansion of PGCLCs. We found that LTC-PGCLCs produce virus-like particles resembling human-infectious retroviruses and that the responsible retrovirus (the HML-2 endogenous retrovirus) is also active in PGCs in human embryos. Testicular cancers are malignancies of PGCs, and these are the most frequent cancers among young men. About 50% of testicular cancer is seminoma, but only one seminoma cell line has ever been established due to technical difficulties. We found that the LTC protocol of PGCLC culture also efficiently supports growth of seminoma cells, and we have successfully established multiple new human seminoma cell lines and associated normal iPS cells from patient-derived tumor tissues. These cell culture resources provide unprecedented opportunities to understand mechanisms of testicular carcinogenesis and vulnerability of PGCs to toxic substances.

Long-term maintenance of human PGCLCs *in vitro*

Several labs, including ours, have established the usefulness of PGCLCs as a cell culture model faithfully resembling human embryonic PGCs. However, PGCLCs are short-lived and lost in cell culture in 10-14 days. This major technical barrier has prevented application of PGCLCs to various studies such as chemical or genetic screenings. To overcome this hurdle, we have performed a systemic evaluation of cell culture conditions and successfully established a novel protocol that supports expansion of human PGCLCs over 100 days without losing their PGC-like characteristics. Under this Long-Term Culture (LTC) protocol, PGCLCs actively migrate and rapidly proliferate without any limit by senescence as telomerase-positive cells while strictly maintaining their PGC-like transcriptomal profile and marker protein expression. The LTC-PGCLC provided us with a unique opportunity to perform proteomics analysis (with Dr. Wilhelm Haas, CCR), which detected retrovirus-like proteins expressed in this cell culture model of normal human PGCs. To our surprise, it turned out that LTC-PGCLCs robustly produce even retrovirus-like particles from their surface. The HML-2 human endogenous retrovirus is responsible for formation of the virus-like particles in PGCLCs, and analysis of previously published single cell RNA-seq data of human embryos revealed HML-2 activation in PGCs *in vivo*. Thus, the LTC-PGCLC model provides the relevant fields of research with unprecedented opportunities to access unlimited amounts of PGCLCs, facilitating studies of normal development and disease formation of human germ cells.

Genetic modeling of human testicular cancers

Testicular cancer is the most common malignancy that affects juvenile and young-adult males at 15-35 years old. The vast majority of testicular cancer is the Type II germ cell tumor, which arise from PGCs, and about 50% of them are seminomas



HML-2 human-specific endogenous retroviruses form virus-like particles at the surface of human primordial germ cell-like cells (hPGCLCs). hPGCLC is a pluripotent stem cell-derived cell culture model of human primordial germ cells, which are the earliest precursor of all germline cells. The viral capsid is assembled beneath the cell surface (center) and eventually pinched out of the cells with plasma membrane surrounding it as viral envelope (right). The virus-like particles are often released from hPGCLCs as aggregates (left).

and the others are non-seminomas such as embryonal carcinomas. Most cases of invasive testicular cancers harbor chromosome (chr) 12p amplification and are associated with Germ Cell Neoplasia In Situ (GCNIS), which consist of cells resembling PGCs and lacking chr12p amplification. Testicular cancer is known for its very strong familial predisposition. Whereas testicular cancers lack genetic mutations commonly found in many other types of adult cancers, they often harbor gain-of-function c-KIT mutations or focal amplification of the gDNA region including the wild type *c-KIT* gene. Genome-wide association studies have repeatedly suggested the involvement of the pro-apoptotic gene *BAK1* in testicular carcinogenesis. However, the mechanisms by which *c-KIT*, *BAK1*, and/or chr12p amplification contribute to testicular carcinogenesis and progression still remain largely unknown due to the lack of adequate experimental models. The genetic basis of the familial predisposition of testicular cancer is also poorly understood. In collaboration with members in Mass General Urology (Keyan Salari, Philip Saylor, Richard Lee) and Urological Pathology (Chin-Lee Wu), we are attempting to make a breakthrough by establishing novel cell lines of human testicular cancers associated with normal iPS cells, from which PGCLCs can be produced. It turned out that out LTC protocol developed for PGCLCs also efficiently support growth of seminomas, which has been represented by only a single cell line (TCam2). We are currently expanding and characterizing multiple cell lines of seminomas, non-seminomas, iPSCs derived from the same testicular cancer patients, and PGCLCs derived from these iPSCs. For example, our T548 embryonal carcinoma cells harbor four extra copies of chr12 and strongly amplified wild type c-KIT, and in direct comparison with the associating normal iPSCs by whole genome sequencing, our T548 embryonal carcinoma cells revealed LOH of loss-of-function CHEK2. Our T836 seminoma cells harbor a gain-of-function c-KIT mutation and amplified ch12p. We are currently working to introduce these prospective driver mutations into the associating normal PGCLCs – which supposedly harbor the unidentified genetic predisposition – to recapitulate the carcinogenic procedure *in vitro*.

Selected Publications:

Lee H, Blumberg B, Lawrence M, **Shioda T**. Revisiting the use of structural similarity index in Hi-C. *Nature Genetics*. 2023. – accepted.

Pierson Smela MD, Kramme CC, Fortuna PRJ, Adams JL, Su AR, Dong E, Kobayashi M, Brixi G, Kavirayuni VS, Tysinger E, Kohman RE, **Shioda** T, Chatterjee P, Church GM. Directed differentiation of human iPSCs to functional ovarian granulosa-like cells via transcription factor overexpression. *eLife*. 2023. 12:E83921.

Kobayashi Mu, Kobayashi Mi, Odajima J, Shioda K, Hwang YS, Sasaki K, Chatterjee P, Kramme C, Kohman RE, Church GM, Loehr AR, Weiss RS, Jüppner, H, Gell JJ, Lau C, and **Shioda T**. Expanding homogenous culture of human primordial germ cell-like cells maintaining germline features without serum or feeder layers. *Stem Cell Reports* 2022 Mar 8;17(3):507-521.

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Shannon Stott, PhD

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**Co-mentored with Michael Lawrence, PhD

The Stott laboratory is comprised of bioengineers, biologists and chemists focused on translating technological advances to relevant applications in clinical medicine. Specifically, we are interested in using microfluidics, imaging, and biopreservation 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) and extracellular vesicles (EVs) from the blood of cancer patients. New microfluidic tools are being developed to both manipulate and interrogate these cells and vesicles at a single particle level. We also look at tumor specimens using multispectral imaging, hoping that the exploration of the spatial relationships between immune cells and tumor tissue will help us better predict treatment response. Ultimately, we hope that by working in close partnership with the clinicians and cell biologists at the Mass General Cancer Center, we can create new tools that directly impact patient care.

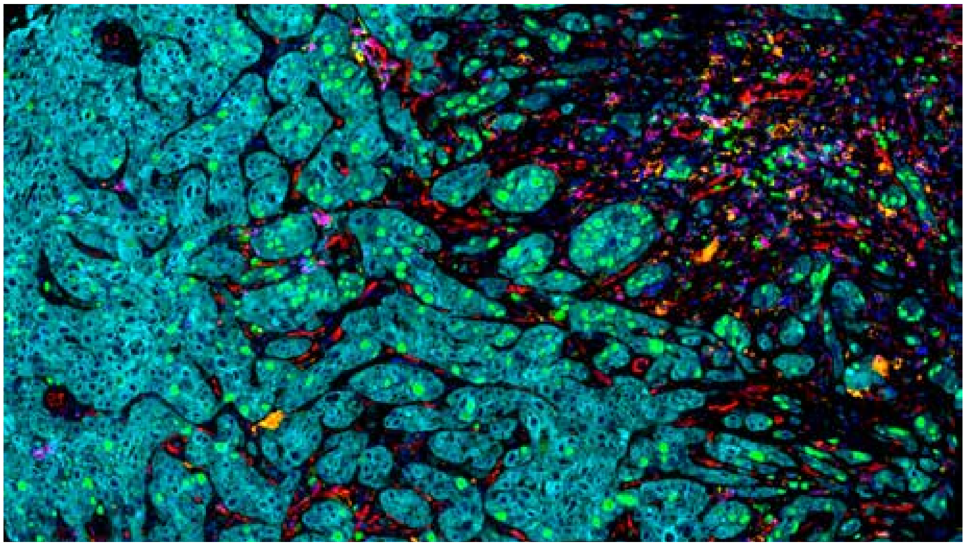
Rapid technological advances in microfluidics, imaging and digital gene-expression profiling are converging to present new capabilities for blood, tissue and single-cell analysis. Our laboratory is interested in taking these advances and creating new technologies to help build understanding of the metastatic 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 at a single cell level, and 3) novel imaging strategies to characterize tumor tissue, cancer cells, 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 cell-specific EVs from 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



Multispectral image of a section of tumor tissue from a patient with head and neck cancer. Various markers were selected for cell identification to explore the relationship between immune cells and cancer cells within the tumor.

Image courtesy of Daniel Ruiz Torres, MD.

high throughput 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 tumor specimens

Tumors can be highly heterogeneous, and their surrounding stroma even more so. Traditionally, the tumor and surrounding cells are dissociated from the tissue matrix for high throughput analysis of each cell. While this allows for important information to be gained, the spatial architecture of the tissue and corresponding interplay between tumor and immune cells can be lost. The Stott lab is developing quantitative, robust analysis for individual cells within the tumor and neighboring tissue using multispectral imaging. We are using this technology alongside downstream imaging processing algorithms to interrogate signaling activity in cancer cells, immune cell infiltration into to the tumor and pEMT 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:

Rabe DC, Ho UK, Choudhury A, Wallace JC, Luciani EG, Lee D, Flynn EA and **Stott SL**, Aryl-Diazonium Salts Offer a Rapid and Cost-Efficient Method to Functionalize Plastic Microfluidic Devices for Increased Immunoaffinity Capture. *Adv. Mater. Technol*. 2300210, 2023.

Rabe DC, Walker ND, Rustandy FD, Wallace J, Lee J, **Stott SL**†, Rosner MR† Tumor Extracellular Vesicles Regulate Macrophage-Driven Metastasis through CCL5. *Cancers*. 13(14): 3459, 2021.

Tessier SN, Bookstaver LD, Angpraseuth C, Stannard CJ, Marques B, Ho UK, Muzikansky A, Aldikacti B, Reategui E, Rabe DC, Toner M, **Stott SL**. Isolation of intact extracellular vesicles from cryopreserved samples. *PLoS One*. 16(5):e0251290, 2021.

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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*. 9(1), 2018.

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Suvà Laboratory

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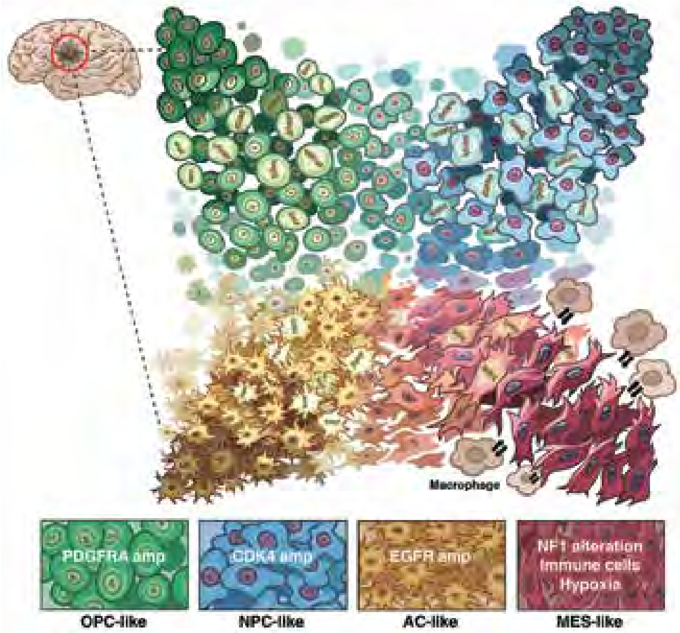
The Suvà laboratory develops and applies single-cell genomic technologies and advanced computational analyses to dissect the biology of brain tumors, in particular adult and pediatric gliomas. We study clinical samples at single-cell resolution and establish genetically and epigenetically faithful cellular models directly from patient 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 subtypes of gliomas, and to leverage the information for renewed therapeutics. In close collaborations, the laboratory additionally leverages single-cell genomics to dissect the immune system of gliomas and to chart the cellular programs in sarcomas

Cell state heterogeneity is an important disease hallmark of both IDH-mutant glioma and IDH-wildtype glioblastoma, with genetic clonal diversity intermingled with neurodevelopmental trajectories. Stemness-to-differentiation diversity is central to the glioma stem cell (GSC) model, which posits that stem-like cells are uniquely capable of self-renewal, tumor propagation and preferential resistance to therapy. Recent single-cell RNA-sequencing efforts in glioma led by my laboratory provided high-resolution mapping of cell state diversity and offered additional granularity to the GSC model by revealing multiple transcriptionally-defined cell states related to neurodevelopmental cell types. Yet, while cellular states can be precisely delineated by scRNAseq, glioma cell state heritability and transition dynamics are not defined, and the epigenetic underpinning of glioma cellular states is still largely unknown. Equally unaddressed are cellular cross-talks within the glioma ecosystem (e.g. cancer-immune interactions). In order to dissect those influences and obtain a comprehensive view of gliomas biology, my laboratory is leveraging joint capture of transcriptional, genetic, and epigenetic information (DNAme, chromatin accessibility) at the single-cell resolution to primary diffuse gliomas. Additionally, we integrate single-cell genomics of human tumors with mouse models, computational deconvolution of profiles from The Cancer Genome Atlas (TCGA) and functional experiments. Our approach offers a compelling framework to comprehensively dissect the glioma ecosystem, both at diagnosis and under therapeutic pressure.

Assessing malignant cells heterogeneity at the single-cell level in gliomas

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. Our work in IDH-mutant gliomas highlighted a rare subpopulation of actively dividing stem/progenitor cells, solely responsible for fueling tumor growth in patients. Single cell profiling of H3K27-mutant pediatric gliomas highlighted specific vulnerabilities and revealed a differentiation block, maybe explaining the more aggressive nature of this cancer type.



Model for the cellular states of glioblastoma and their genetic and micro-environmental determinants. Mitotic spindles indicate cycling cells. Lighter/ darker tones indicate strength of each program. Intermediate states are shown in between the four states and indicate transitions.

More recently, we provided a comprehensive model of glioblastoma biology that integrates single-cell expression programs, genetic composition and tumor subtypes (see figure). Our study of medulloblastoma single-cell programs provided clarifications on tumor histogenesis and classification. The lab is currently performing such single-cell analyses with constantly increased throughput, resolution and in broader clinical settings (e.g. rare entities, novel clinical trials). Overall, our goal is to identify both lineage-defined and somatically-altered therapeutic targets in brain cancer in both children and adults.

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, 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:

Hara T†, Chanoch-Myers R†, Mathewson ND, Myskiw C, Fan J, Bussema L, Atta L, Eichhorn SW, Greenwald A, Kinker G,…Hunter T, Verma IM, Wucherpfennig KW, Regev A, **Suvà ML***, Tirosh I*. Interactions between cancer cells and immune cells drive transitions to mesenchymal-like states in glioblastoma. *Cancer Cell.* 2021 Jun 14;39(6):779-792.

Mathewson ND†, Ashenberg O†, Tirosh I†, Gritsch S†, Perez EM†, Marx S†, Jerby-Arnon L, Chanoch-Myers R, Hara T,…Louis DN, Ligon KL, Marson A, Chiocca EA, Reardon DA, Regev A*, **Suvà ML***, Wucherpfennig KW*. Inhibitory CD161 Receptor Identified in Glioma-infiltrating T cells by Single Cell Analysis. *Cell.* 2021 Mar 4;184(5):1281-1298.

Neftel C†, Laffy J†, Filbin MG†, Hara T†, Shore ME, Rahme GJ, Richman AR, Silverbush D, Shaw ML, Hebert CM, Dewitt J, Gritsch S, Perez L, Gonzalez Castro LN,…Louis DN, Regev A, Bernstein BE, Tirosh I*, **Suvà ML***. An integrative model of cellular states, plasticity and genetics for glioblastoma. *Cell.* 2019 Aug 8;178(4).

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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David A. Sweetser, MD, PhD

Sweetser Laboratory

David A. Sweetser, MD, PhD
Evangelos Theodorou, PhD

The Sweetser laboratory investigates how leukemia and other cancers form with the goal of developing novel, safer, and more effective therapies. Our lab has identified a novel family of tumor suppressor genes, the Groucho/TLE family of co-repressors and defined how TLE1 and TLE4 function as potent tumor suppressors of acute myeloid leukemia and how they have critical roles in hematopoiesis, bone, lung, and brain development, and limiting inflammation. It is this latter function that appears to underlie their tumor suppressor role. Currently, we are defining a cooperative role of TLE1 in melanoma development. A second line of research seeks to define and target critical signaling pathways within the cancer niche that are required for the proliferation and survival of leukemia. As the Mass General site director for the Undiagnosed Diseases Network and Chief of Medical Genetics and Metabolism at Mass General, 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.

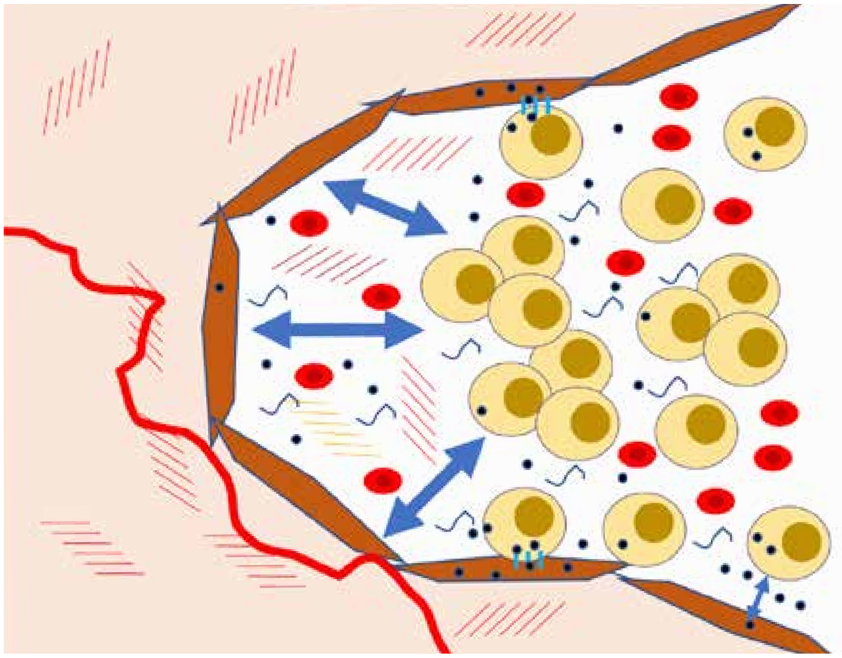
Evaluation of the role of the Groucho/TLE family of corepressors in cancer and development

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 and other cancers.

The Groucho/TLE family of corepressor proteins can modulate many of the major pathways involved in development and oncogenesis, including Wnt/β-catenin, Notch, Myc, NFκB, and TGFβ. These genes appear to behave as tumor suppressor genes in the pathogenesis of other myeloid malignancies and lymphomas, but as an oncogene in synovial cell sarcoma. 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. Our work indicates this cooperative effect appears to involve regulation of Wnt signaling and inflammatory gene pathways. This work has led to the demonstration that specific anti-inflammatory agents can have potent anti-leukemic effects. We are currently studying the role of TLE1 in melanomas using conditional knockout of Tle1 and conditional oncogenicV600E BRAF expression.

The role of the bone marrow niche in nurturing leukemia

The bone marrow niche is remodeled in the process of leukemia development to provide a supportive environment that contributes to leukemic cell proliferation, survival, and resistance to chemotherapy. Leukemia treatments to date have focused on attacking leukemia cells and have largely ignored that fact that the survival of leukemia is critically dependent on the supportive role of a transformed leukemic bone marrow niche. This bone marrow niche is rich in cytokines, growth factors, and various nucleic acids including miRNAs. Using diagnostic bone marrow aspirates from patients with leukemia and controls



Schematic diagram of the leukemic bone marrow niche. *Remodeling of the bone marrow niche creates a necessary and supportive environment for the development and expansion of leukemia. This synergistic cross talk involves a complex milieu of compounds including cytokines, growth factors, miRNAs and other nucleic acids and proteins. Disruption of critical signals in this niche could represent a valuable therapeutic strategy.*

we have characterized many of these dysregulated components in bone marrow stroma, bone marrow plasma and leukemic cells. We are now systematically evaluating these to identify novel therapeutic modalities to block critical signals necessary to sustain leukemic growth and survival.

The undiagnosed diseases network

Dr. Sweetser is also engaged in rare and undiagnosed disease research. The Harvard Medical School Hospital consortium of Mass General, Brigham and Women's Hospital and Children's Hospital together with 11 other clinical sites around the US comprise the NIH sponsored Undiagnosed Diseases Network. As Chief of Medical Genetics at Mass General, and the Mass General site director for the UDN, Dr. Sweetser coordinates a team of expert clinicians and researchers, using comprehensive clinical phenotyping, whole exome/whole genome sequencing, paired with RNASeq and metabolomics profiling, in vitro functional modeling, and collaboration with zebrafish and Drosophila model organism cores to identify the underlying basis of a variety of challenging human diseases. Over three dozen new genetic disorders have been characterized with these efforts. His lab is also developing stem cell models of several inherited neurological disorders to understand alterations in brain development and potential novel therapies.

Selected Publications:

Galazo M, **Sweetser DA**, D. Macklis J. Tle4 controls both developmental acquisition and postnatal maintenance of corticothalamic projection neuron identity. May 2022 *BioRxiv*. https://doi.org/10.1101/2022.05.09.491192

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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.

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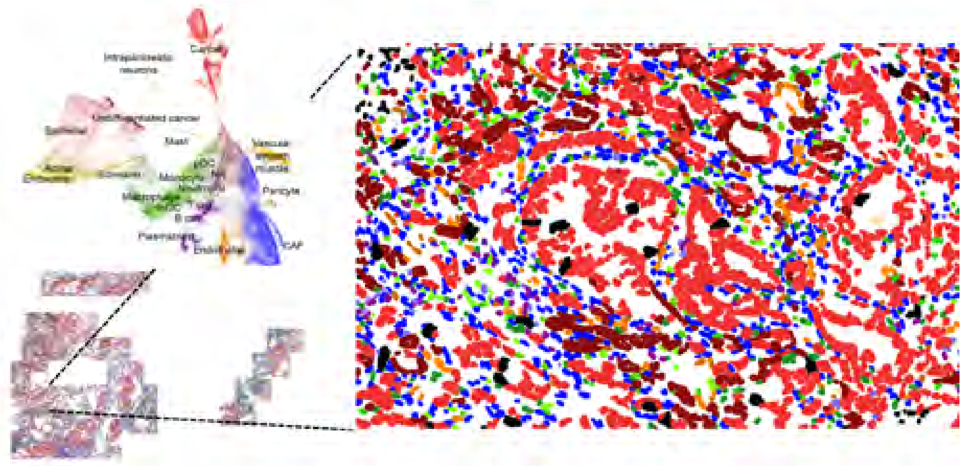
Gastrointestinal cancers are highly lethal cancers where the vast majority of patients are diagnosed too late and conventional therapies have largely been ineffective, making early detection and novel drug targets greatly needed. **The Ting laboratory** has been utilizing innovative technologies to characterize RNA expression patterns in cancer. Using single molecule sequencing, we have discovered a significant amount of "non-coding" repeat RNAs to be produced in high amounts at the earliest stages of cancer development, but not in normal tissues. These repeat RNAs can serve as a novel early detection cancer biomarker and they can be targeted as a new therapeutic avenue. In parallel, we have used single cell, microfluidic chip technologies, and spatial transcriptomics to understand the factors involved in cellular plasticity in cancer. Our studies in circulating tumor cells (CTCs) have revealed the importance of tumor-stromal crosstalk in pancreatic cancer. We are using these studies to generate new therapies to stop the metastatic spread of cancer.

The Ting laboratory has utilized RNA-sequencing, RNA in situ hybridization, and spatial transcriptomic technologies 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 used single cell, spatial transcriptomic, and microfluidic technologies to understand cancer cell heterogeneity and plasticity. We have now found the aberrant expression of repeat RNAs are linked with cancer cell plasticity.

Repeat non-coding RNAs

RNA sequencing of a broad spectrum of carcinomas demonstrated a highly aberrant expression of non-coding repeat RNAs emanating from regions of the genome previously thought to be inactive due to epigenetic silencing. Analysis of all human repeats 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, our work has found that these satellite repeats can affect the local tumor microenvironment with implications for immunotherapies.

This has led to a Phase II clinical trial of a nucleoside reverse transcriptase inhibitor (NRTI) 3TC in metastatic colorectal cancer, which is demonstrating promising single agent activity in 25% of patients. We are now trying to identify the HSATII reverse transcriptase and better understand the biological role of satellites in cancer progression and tumor immune response.



This image represents a spatial transcriptomic "map" of a pancreatic cancer with individual molecules of repeat and coding RNAs quantified with precise spatial coordinates in a human primary tumor sample. Individual cell types can be determined based on transcriptional profiles with mapping to understand cell-cell interactions within tissue.

Pancreatic cancer cellular heterogeneity

The high lethality of pancreatic cancer results from an intrinsic ability to resist chemotherapy and the propensity to metastasize. The etiology of this behavior is multifactorial, but our group has identified cancer cell heterogeneity and plasticity as key elements of aggressive pancreatic cancer. Our initial work using a microfluidic device to isolate rare circulating tumor cells (CTCs) offered a window into understanding the metastatic cascade. These studies demonstrated the inherent heterogeneity of pancreatic CTCs and their ability to seed metastases through a partial epithelial mesenchymal transition (EMT) program. We have recently uncovered the importance of stromal cancer associated fibroblasts (CAFs) in inducing EMT single cell heterogeneity consistent with phenotypes observed in CTCs and the plasticity of EMT phenotypes in the setting of chemoresistance and metastasis. Moreover, we defined pancreatic cancer intratumoral heterogeneity in discrete tumor glands using RNA-ISH and high content digital image analysis. We are now using spatial transcriptomic methods to fully characterize the relationship of tumor cell plasticity and CAF heterogeneity. In addition, this platform provides a strategy to understand the spatial relationship of these cell types important for pancreatic cancer pathogenesis. More recently, we have now identified repeat RNA intercellular delivery via extracellular vesicles as a mechanism to drive cellular heterogeneity in pancreatic cancer. The understanding of cellular crosstalk of pancreatic cancer cells with CAFs and other microenvironmental cells will provide new mechanistic insight in the drivers of cancer cell heterogeneity and CTC generation, identify biomarkers in predicting patient outcomes, and reveal novel therapeutic avenues targeting tumor cell microenvironment interactions.

Selected Publications:

Porter RL*, Sun S*, Flores MN, Berzolla E, You E, Phillips IE, Kc N, Desai N, [...] Stott SL, Deshpande V, Liu JF, Solovyov A, Matulonis UA, Greenbaum BD†, and **Ting DT**†. Satellite repeat RNA expression in epithelial ovarian cancer associates with a tumor-immunosuppressive phenotype. *J Clin Invest*, 2022 Aug 15;132(16):e155931.

Rajurkar M*, Parikh AR*, Solovyov A*, You E, Kulkarni AS, Chu C, Xu KH, Jaicks C, Taylor MS, Wu C, Alexander KA, Good CR, Szabolcs A, Gerstberger S, [...] Deshpande V, Rivera MN, Aryee MJ, Hong TS, Berger SL, Walt DR, Burns KH, Park PJ, Greenbaum BD†, and **Ting DT**†, Reverse Transcriptase Inhibition Disrupts Repeat Element Life Cycle in Colorectal Cancer. *Cancer Discovery*, (2022).

Parikh AR*, Szabolcs A*, Allen JN, Clark JW, Wo JY, Raabe M, Thel H, Hoyos D, Mehta A, [...] Greenbaum BD, **Ting DT**†, and Hong TS†, Radiation therapy enhances immunotherapy response in microsatellite stable colorectal and pancreatic adenocarcinoma in a phase II trial. *Nat Cancer*, (2021); 2(11): 1124-1135.

Franses JW*, Philipp J*, Missios P, Bhan I, Liu A, Yashaswini C, Tai E, [...] Ryan DP, Maheswaran S, Haber DA, Daley GQ, and **Ting DT**. Pancreatic circulating tumor cell profiling identifies LIN28B as a metastasis driver and drug target. *Nature Communications* (2020); 11(1): 3303.

Porter RL, Magnus NKC, Thapar V, Morris R, Szabolcs A, Neyaz A, Kulkarni AS, Tai E, [...] Ferrone CR, Haber DA, and **Ting DT**. Epithelial to mesenchymal plasticity and differential response to therapies in pancreatic ductal adenocarcinoma. *PNAS* (2019); 116(52): 26835-26845.

Ligorio M*, Sil S*, Malagnon-Lopez J, Nieman LT, [...] Fernandez-Del Castillo C, Ferrone CR, Haas W, Aryee M†, **Ting DT**†. Stromal Microenvironment Shapes the Intratumoral Architecture of Pancreatic Cancer. *Cell*. (2019 Jun 27); 178(1):160-175.e27.

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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. These control vital genes in cancer and are important for chemoresistance and 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

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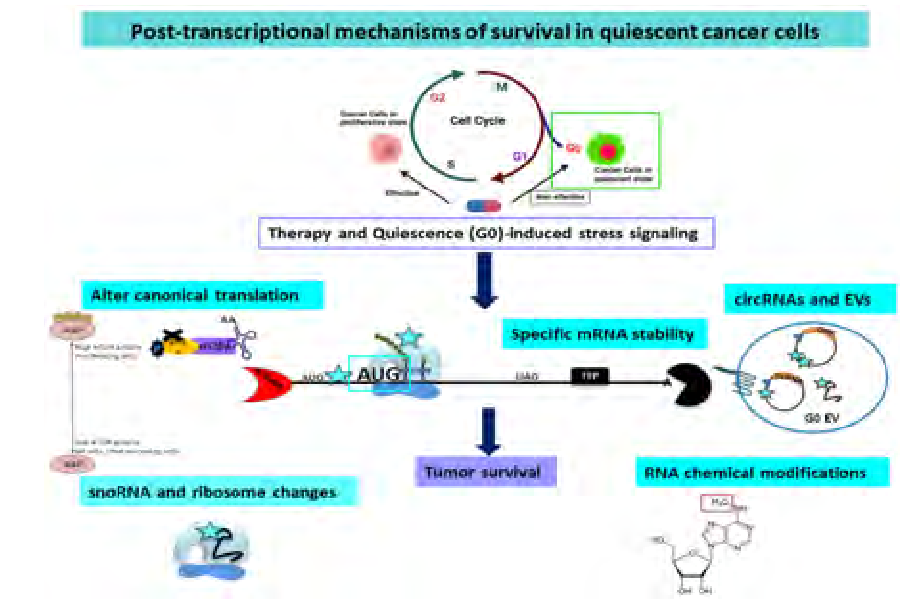
Quiescent (G0) cells are observed as a clinically relevant population in leukemias and other tumors associated with poor survival. G0 is a unique, nonproliferative phase that provides an advantageous escape from harsh situations like chemotherapy, allowing cells to evade permanent outcomes of senescence, 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 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



Targeting RNA mechanisms of tumor persistence

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:

Datta C, Truesdell SS, Wu KQ, Bukhari SIA, Ngue H, Buchanan B, Le Tonqueze O, Lee S, Kollu S, Granovetter MA, Boukhali M, Kreuzer J, Batool MS, Balaj L, Haas W, **Vasudevan S**. Ribosome changes reprogram translation for chemosurvival in G0 leukemic cells. *Sci Adv*. 2022 Oct 28;8(43):eabo1304.

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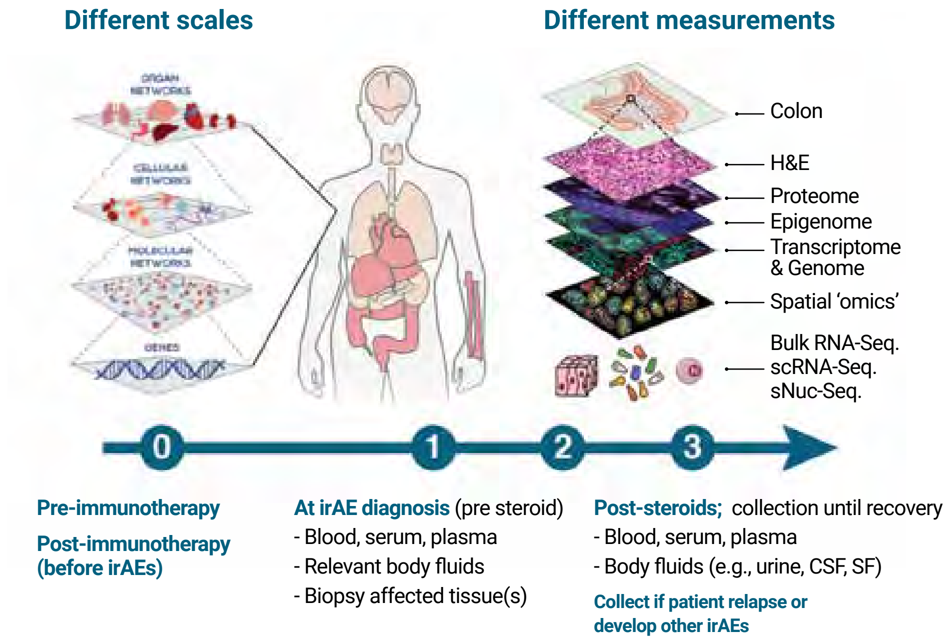
The Villani laboratory seeks to establish a comprehensive roadmap of the human immune system by achieving a higher resolution definition and functional characterization of cell subsets and rules governing immune response regulation, as a foundation to decipher how immunity is dysregulated in diseases. 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.

Leveraging single-cell 'omics' to unravel new insights into the 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) and 'multi-omics' 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 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



Overview of our strategy for exploring scale, time and modalities to discover underpinnings of diseases.

of in-depth expertise in single-cell 'multi-omics' experimental and computational strategies (Ding , *Nat Biotechnol* 2020; Li, *Nat Methods* 2020; Tukiainen, Villani, *Nature* 2017; Ranu, Villani, *Nucleic Acid Res* 2019; Villani, *Methods Mol Biol* 2016), and its application to study immune cells infiltrates in healthy, tumor lesions and inflamed tissue (Izar, *Science* 2016; Sade-Feldman, *Cell* 2019; Di Pilato, *Nature* 2019; Olah, *Nat Commun* 2018; Balan, *Cell Rep* 2018; Popescu, *Nature* 2019; Smillie, *Cell* 2019; Abbas, *Nat Immunol* 2020; Delorey, *Nature* 2021; Alladina, *Science Immunol* 2023).

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 irAE incidence 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 (Zubiri, *J Immunother Cancer* 2021; Thomas, *Nature Med* 2023). 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, and/or (ii) targeted after onset of irAEs, without reducing the efficacy of the immunotherapy.

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Publications September 1, 2022 – August 31, 2023

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