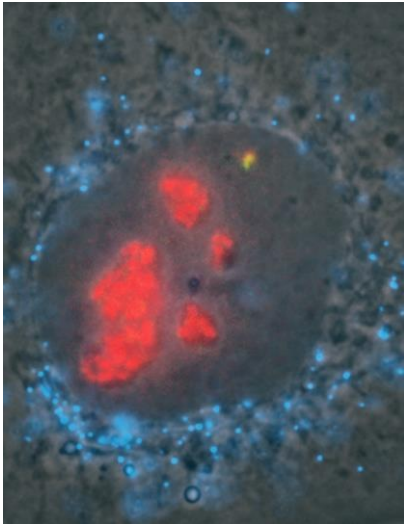
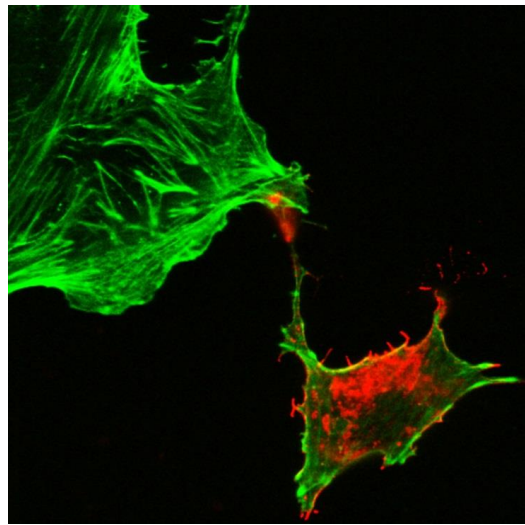


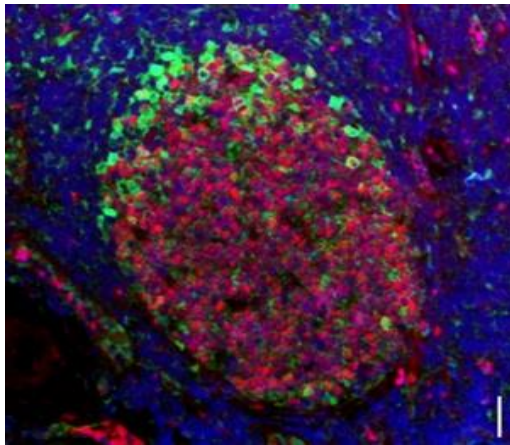
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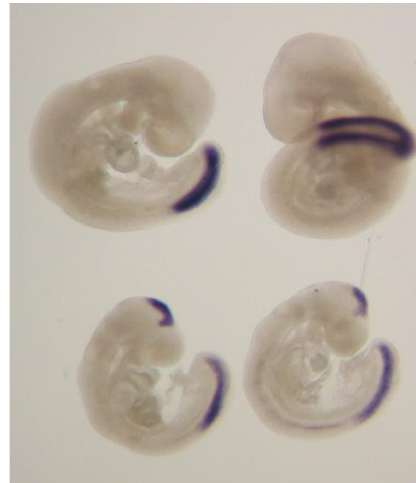
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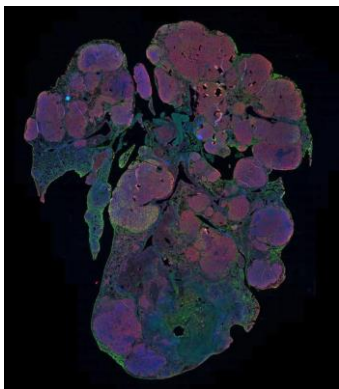
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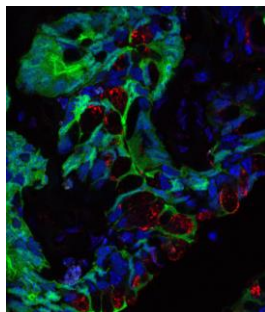
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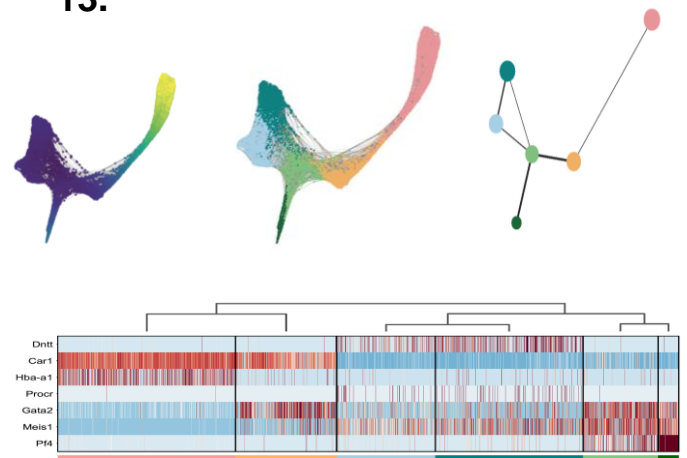
11.



12.



13.



## Picture Legends

1. **Query Lab:** Scheme for progression of pre-mRNA splicing, highlighting the different conformations required for the first and second catalytic steps; modulation of transition between the two catalytic steps by spliceosomal mutations results in altered splicing fidelity and splice site choice.
2. **Fyodorov & Skoultschi Labs:** *Drosophila melanogaster*, a model system to study biochemistry and genetics of chromatin.
3. **Gritsman Lab:** Spectral karyotyping analysis revealing multiple chromosomal abnormalities in hematopoietic cells from a PI3 kinase (PI3K) triple isoform knockout (TKO) mouse
4. **Schildkraut Lab:** Fluorescent antibodies showing replication fork direction in single DNA molecules labeled with halogenated nucleotides.
5. **Skoultschi Lab:** ChIP-Seq – Chromatin immunoprecipitation followed by massive parallel sequencing reveals differences in the DNA binding patterns of transcription factor PU.1 in normal red blood cells (ES-EP) and malignant erythroleukemia cells (MEL).
6. **Kitsis Lab:** Cell death. Healthy (left) and dying (right) HEK293 cells. Blue - Hoechst 33342 staining of nuclei. Red-tetramethyl rhodamine ethyl ester reflecting electrical potential difference across the inner mitochondrial membrane.
7. **Meier Lab:** Fluorescence image overlayed on a phase contrast image of an enlarged nucleus surrounded by cytoplasm. This stable cell line expresses a cyan fluorescent protein (CFP) with a peroxisomal targeting signal (blue) and a small nucleolar RNA (snoRNA, red) from an intron (green). In this fluorescence in situ hybridization (FISH) of RNA, the snoRNA is visualized in nucleoli (red) and together with the intron at the site of transcription (green/yellow). The spliced mRNA is exported to the cytoplasm and the translated CFP translocated into peroxisomes (blue).
8. **Ye Lab:** Immunofluorescence staining reveals that transcription factors BCL6 (red) and STAT3 (green) are expressed in separate populations of B cells within the germinal center, a dynamic microenvironment critical for T-cell dependent antibody response.
9. **Kielian lab:** Alphavirus-infected cells showing intercellular extensions that mediate virus cell-to-cell transmission. Confocal microscopy image of alphavirus-infected Vero cells, with virus glycoproteins shown in red and F-actin shown in green.
10. **Stanley Lab:** The Notch ligand Dll3 is upregulated in mid-hind brain of E8.5 mouse embryos lacking *O*-fucose glycans on Notch receptors.

11. **LaFave Lab:** Multiplexed immunohistochemistry in a murine model with advanced lung adenocarcinoma. Whole tumor-burdened lungs were stained for Alveolar type 2 (AT2) (OPAL 520, green), Alveolar type 1 (AT1) (OPAL 690, red), and nuclear (DAPI) markers. Image generated by the LaFave lab.

12. **Edelmann Lab:** Lgr5<sup>+</sup> and Paneth cells form a stem cell niche in MMR-deficient intestinal tumors

13. **Steidl Lab:** Single-cell RNA-sequencing of mouse hematopoietic stem and progenitor cells reveals a continuum of cells differentiating into megakaryocytic and erythroid lineages. (Left) A force-directed graph colored by pseudotime. (Middle) Community detection via the Louvain method. (Right) Partition-based graph abstraction (PAGA) provides an interpretable graph-like map of the arising data manifold. (Bottom) Cell-type-specific markers aid in cluster identification. [Emily Schwenger, MSTP student, Steidl Laboratory]

## **Department of Cell Biology**

Welcome to the Albert Einstein College of Medicine and the Department of Cell Biology. Our department is focused on molecular mechanisms in many important areas of cell biology, ranging from stem cells to viruses, DNA replication to RNA processing, gene expression to immunology, glycobiology to cancer. We share many common interests and enjoy an interactive and scientifically stimulating atmosphere that makes the Cell Biology Department a great place to work.

Graduate students in Cell Biology participate in a variety of departmental activities. As needed, these are online get togethers during the pandemic. The department meets every Friday for a “work-in-progress” seminar in which post-doctoral fellows and graduate students describe the progress of their current research and discuss future directions. The department hosts a bi-weekly seminar program of invited outside speakers, with many opportunities for students and postdocs to meet the speaker for discussion and lunch. There is a departmental journal club series in which students present original articles and discuss over dinner. A Friday afternoon get-together encourages scientific interactions as well as social connections. Every few years, our departmental retreat takes us all to the seashore or mountains for a chance to talk about the big picture of our research, to enjoy poster presentations from students and postdocs, and to try to solve the zany puzzles organized by the skit committee.

On the following pages you will find information about the research programs of the individual faculty members, as well as listings of the current students and postdocs in the department. You can also find out more about the department on our web page at <http://www.einstein.yu.edu/cellbiology> . Feel free to contact any of us for further discussions.

Enjoy your first year!



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Co-Leader Tumor Microenvironment and Metastasis Program, AECC



Price BLDG. – ROOM 220

**CANCER DORMANCY: UNDERSTANDING THE BIOLOGY OF METASTASIS**

The major challenge faced by physicians is the prevention and treatment of metastasis, the main reason for cancer mortality. Often, cancer patients presumed cured after primary tumor removal and therapy, can carry non-proliferating ‘dormant’ disseminated cancer cells (DCCs) for years before reactivating to form incurable metastasis. In addition, DCCs show resistance to standard treatments by reprogramming themselves in a niche-dependent manner. Our lab specifically focused on the dormant nature of minimal residual disease in cancer by understanding the biology of dormant DCCs and their reactivation, to target them and prevent relapse. We focus on identifying the mechanisms of microenvironmental cues and cross-talk with DCCs, epigenetic and immune regulation of DCCs, and developing therapeutic approaches to target DCCs clinically.

- 1. Microenvironmental and epigenetic control of dormancy and metastasis.** We explored why in many patients metastasis remain dormant in the bone marrow. We found that the bone marrow contains high levels of TGF $\beta$ 2, which induced dormancy via TGF $\beta$ -RI/RII/RIII complexes and p38 signaling. Using mouse genetics, we showed that a key source of TGF $\beta$ 2 is the NG2+/Nestin+ mesenchymal stem cells (MSCs) that induce hematopoietic stem cell dormancy and self-renewal and also proved that these MSCs induce and maintain dormancy of breast cancer DCCs in the bone marrow. Analysis of the transcriptional and epigenetic mechanisms active in dormant DCCs pinpointed *retinoic acid* as a micro-environmental pro-dormancy cue. We found that the orphan nuclear receptor NR2F1, which regulates lineage commitment and is silenced in human tumors, is spontaneously upregulated in solitary dormant tumor cells. Retinoic acid induces TGF $\beta$ 2, NR2F1 expression and dormancy of DCCs. We found that a transient treatment with low dose of 5-azacytidine, followed by *all-trans* retinoic acid, restored the NR2F1-driven program and long-term *in vivo* quiescence of previously malignant cells. Our work revealed markers that predict for longer metastasis-free periods in ER+ breast cancer patients. These signatures can be found in single DCCs from prostate cancer patients in clinical dormancy for up to 18 years and not in DCCs from patients with advanced disease.
  - Bragado, P., et al.,(2013). **Nat Cell Biol** PMID 24161934
  - Sosa, M.S., et al.,(2015). **Nat Commun** PMID 25636082
  - Nobre et al., (2021) **Nat Cancer** PMID 34993493
  - Borriello et al., (2022) **Nat Commun** PMID 35110548
  - Khalil et al., (2022). **J. Exp. Med.** PMID 34812843
  - Carcamo et al., (2022) **Cell Reports**. PMID 35385731
  - Fane et al., (2022) **Nature** PMID 35650435
- 2. Early dissemination and early DCC dormancy.** Our lab has discovered that dormant breast cancer DCCs and metastasis can originate very early during cancer evolution, disseminating during pre-malignant stages and aided by innate immune cells. We identified a mechanism for early dissemination whereby Her2 aberrantly activates a program similar to mammary ductal branching that spawns early

DCCs (eDCCs) capable of forming metastasis after a dormancy phase. We also revealed how the HER2 oncogene activates through CCL2 signaling the recruitment of tissue resident macrophages that help eDCCs to enter circulation. Targeting these macrophages early in cancer evolution reduced metastasis late in cancer progression. Our focus is to understand how eDCCs found metastasis directly and/or through the preparation of eDCC-orchestrated pre-metastatic niches for later arriving DCCs to colonize target organs. We also aim to identify markers that might pinpoint early DCCs vs late DCCs and that may allow selectively targeting these cells.

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- Hosseini et al., (2016) **Nature**. PMID 27974799
- Linde and Casanova-Acebes et al., (2018) **Nat Commun**. PMID 29295986
- Nobre et al., (2022) **Nat Cancer** (In press) Pre- print doi: 10.21203/rs.3.rs-145308/v1

3. **The balance between mitogenic and stress signaling as a determinant of dormancy.** We found that the ERK to p38 ratio was predictive of cancer cell dormancy or reactivation across different cancers, including HNSCC, breast and prostate. Our lab discovered that all cancer cells activate p38 upon dissemination but those that go on to metastasize from DCCs silence p38 signaling. We also found that p38 induced in dormant cells an unfolded protein response, upregulation of the chaperone BiP and activation of the ER kinase PERK. We also discovered that stress signaling activated by hypoxia in primary tumor microenvironments gives rise to a sub-population of dormant DCCs that evade therapy and may be the source of disease relapse and poor prognosis associated with hypoxia. We also discovered that the composition and organization of collagen molecules can regulate the decision between dormancy and reactivation by modulating mitogenic and stress signaling pathways.

- Aguirre-Ghiso, J.A., et al., (2004). **Cancer Res**. PMID 15492254
- Schewe, D.M., and Aguirre-Ghiso, J.A. (2008). **PNAS USA**. PMID 18650380
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- Fluegen et al., (2017) **Nat Cell Bio** PMID 28114271
- DiMartino et al., (2022) **Nat Cancer** PMID 35121989

4. **Adhesion, stress signaling and autophagy.** We found that p38 is activated upon loss of integrin adhesion signaling leading to ERK1/2 inhibition and induction of the pro-apoptotic protein BimEL. This is key to induce anoikis, proper development of the mammary tree. We found that the HER2 oncogene inhibits p38 to accelerate cancer progression via anoikis resistance. We also discovered that proper adhesion signaling limits activation of the endoplasmic reticulum PERK, which limits mammary cancer initiation by blocking proliferation. We found that ErbB2 signaling is dependent on optimal activation of eIF2 $\alpha$  signaling and that causing an imbalance in P-eIF2 $\alpha$  levels killed ErbB2-overexpressing cells. We also showed that PERK-eIF2 $\alpha$ -ATF4-CHOP activation in ECM-detached mammary epithelial cells induces autophagy and antioxidant responses for survival.

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- Avivar-Valderas A et al., (2013). **Oncogene**. PMID 23160380

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**Key Words:** *intestinal homeostasis and cancer, diet, mouse models, inflammation, stem cells*

Our research is on the cellular and molecular mechanisms that maintain intestinal homeostasis, and perturbations that disrupt this causing intestinal and colonic disease - in particular, tumor development. This has involved development of mouse genetic models targeting intestinal cell maturation and lineage specific differentiation, and the impact of environmental alterations which in human populations are linked to altering probability of tumor development.

We demonstrated an orchestrated reprogramming of intestinal epithelial cells as they migrate from the progenitor cell compartment in the intestinal crypt and undergo maturation, eliminating cell cycling and promoting cell differentiation. We determined where key regulators of proliferation are active during this traverse of cells along the crypt-luminal axis, their oscillation along this axis, and effects of their disruption on mucosal homeostasis and tumor development.

### **The Profound Role of Diet in Both Genetically Initiated and Sporadic Tumorigenesis:**

Colon cancer incidence in human populations is strongly linked to long term dietary patterns. For the mouse, this can be modeled using a purified rodent diet (NWD1). NWD1 incorporates changes of a number of key nutrients to mimic the level of intake of each to its level consumed in western populations with a much higher incidence of colorectal cancer. Feeding NWD1 accelerates and increases tumorigenesis in mouse genetic models of intestinal cancer, regardless of etiology, mechanism, or altered genetic drivers. For example, we collaborated with Winfried Edelmann on a mouse he engineered to be a faith-full genetic model for Hereditary Non-Polyposis Colon Cancer (HNPCC, or Lynch Syndrome). Feeding these mice NWD1 caused a major increase of tumor development in the large intestine, which is the site-specificity characteristic of individuals in human pedigrees who are carriers of inherited genetic mutations causal for Lynch Syndrome.

However, the vast majority (~80%) of human colon tumors are sporadic, developing without known inherited genetic factors, and arising after 5-6 decades of life with incidence largely determined by long term dietary patterns. Importantly, therefore, feeding NWD1 to wild-type C57Bl6 mice for 1-2 years caused sporadic small and large intestinal tumors with a lag, incidence, frequency and pathology similar to that of *sporadic* colon cancer in the human. This is the only mouse model of common sporadic tumors. Long before tumors developed, the mucosa, though seeming to function normally, exhibits distinct alterations at the cellular and molecular levels: there is macrophage infiltration into the mucosa, elevated serum cytokine levels, decreased secretory and increased absorptive cell lineage marker expression; and elevated Wnt signaling and ectopic Paneth cell marker expression throughout the small intestinal and colonic mucosa.

These discoveries led to our current research on interaction of genetic and nutritional factors in the function of Lgr5<sup>hi</sup> intestinal stem cells. In summary, feeding the NWD1 (higher in fat, lower in vitamin D<sub>3</sub>, calcium, methyl donors and fiber), decreases the ability of Lgr5<sup>hi</sup> intestinal cells to function as stem cells in both intestinal homeostasis and in tumor development. An important role of lowered Vitamin D signaling in this was established in recapitulating the phenotype by targeted inactivation of the vitamin D receptor targeted to Lgr5<sup>hi</sup> cells. The dietary effect is linked to extensive transcriptional reprogramming of the Lgr5<sup>hi</sup> cells. For example, expression of the DNA mismatch repair pathway is elevated by lower vitamin D<sub>3</sub> and/or calcium in the diet, paralleled by reduced accumulation of relevant somatic mutations detected by single Lgr5<sup>hi</sup> cell whole exome sequencing. In compensation, NWD1 also reprograms a second cell population - Bmi1+ cells - to function and persist as stem-like cells in mucosal homeostasis and tumor development. There is also a key role of compromised mitochondrial structure and function in inhibiting ability of Lgr5<sup>hi</sup> cells to function as stem cells, linked to down-regulation of Ppargc1a, a master regulator of Ppar signaling and mitochondrial biogenesis.

Key questions raised by the data are under investigation: **a)** What are the signals, and how are they transmitted, that recruit the Bmi1+ cells to function as stem cells? **b)** Is there a specific subpopulation of the heterogeneous Bmi1+ cell population that is mobilized, and what is its molecular phenotype (under investigation by single cell RNAseq). **c)** Since human diets vary considerably, how does this impact which and how different potential intestinal stem cell populations function in the population and can give rise to tumors? Further, are genetic, epigenetic and clinical phenotypes of colon tumors a function of this nutritional impact on stem cell populations? And what is the implication of nutritionally linked genetic and epigenetic alterations in relatively long-lived stem cells for effective approaches for prevention? **d)** Finally, experimentally induced damage to Lgr5<sup>hi</sup> cells can recruit multiple different intestinal cell populations to function as stem cells; our data demonstrate that this plasticity of function can also be marshalled by nutritional alterations. In this context, “*adaptive radiation*” – the rapid expansion of species and higher taxa to occupy new niches – is driven principally by ability of organisms to adapt to and utilize new sources of food. Therefore, an interesting hypothesis is that the plasticity of intestinal epithelial cells to function as stem cells may have arisen to provide flexibility to organisms to function in new ecosystems.



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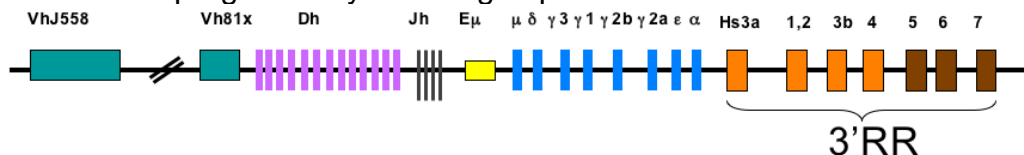
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## **Regulation of Antibody Heavy Chain Gene Rearrangements and Expression by the 3' Regulatory Region**

The immune system is our spacesuit for life in an environment containing enormous numbers of infectious agents. An essential part of the immune system is the B cell, which is the only cell type that produces antibodies. Antibody (Ig) genes are constructed via a series of DNA rearrangements. Occasionally, mistakes occur during antibody construction, which activate oncogenes and lead to cancers. My long term goal has been to understand the mechanisms that initiate and control antibody gene rearrangements within the 3 megabase heavy chain gene (IgH) locus.

Our experiments have focused on a complex 3' IgH regulatory region (3' RR) that lies immediately downstream of the antibody heavy chain gene cluster in mouse and humans. This region has recently been identified as a "super-enhancer" by Richard Young's laboratory, which activates the myc oncogene when it comes under its control as a result of chromosomal translocation. The ~50 kb 3' RR containing multiple regulatory elements, a 1 kb intronic enhancer, and two small elements in the diversity DH gene region are the only currently known long-range regulatory regions for the IgH locus. We have identified an extension of the 3' RR (hs5, 6, 7, 8), which like the other 3' enhancers, has binding sites for Pax5, a transcription factor essential for B cell development. In addition, the 3' RR extension contains insulator activity, i.e. prevention of communication between an enhancer and its target promoter. This is associated with multiple binding sites for CTCF, a protein identified in all mammalian insulators. Chromatin analysis shows that the 3' RR extension is likely to be active throughout B cell development while other 3' RR segments become progressively and stage-specific active.



Murine germline Igh locus, with variable (VH), diversity (DH), joining (JH) and constant region genes (□u, □delta, □amma, □psilon, □lpha). Regulatory elements, Eμ and the 3' regulatory region (3'RR), are depicted. Two CTCF sites in the DH segment are important for VDJ joining.

The 3' RR has been shown to be critical both for class switch recombination that affects expression of virtually all antibody classes and for somatic hypermutation of the heavy chain variable region. In addition, the 3' RR regulates high levels of expression of antibodies in fully differentiated plasma cells. A major goal of our laboratory has been to understand the mechanisms by which the 3' RR functions normally in both mouse and human, both by acting on the Igh locus and by insulating the locus from its non-Igh neighboring genes. CTCF binding sites are anticipated to play a major role in these activities by promoting interactions between distal DNA sequences through loop formation.

We have been analyzing the extent of B cell-specific regulation of the IgH locus, by studying DNA demethylation, histone modifications of and the binding of CTCF and Pax5, and other factors to 3' RR sequences during B cell development and class switching.. Furthermore, we have examined the interaction of the 3' RR with target sequences within the IgH locus, as assessed by the

chromosome capture conformation technique (3C). Together, these approaches have been designed to understand how the 3' RR facilitates molecular acrobatics necessary for the immunoglobulin heavy chain locus without their intrusion on downstream non-IgH genes, or resulting in chromosomal translocations involved in malignancies.

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**Key Words:** *Pluripotent Stem Cells, Hematopoietic Stem Cells Red Blood Cells, Sickle Cell Disease, Gene Therapy, Genome Modification.*

#### **CULTURED RED BLOOD CELLS:**

We have developed a method to produce genetically engineered human red blood cells by differentiation of human induced-Pluripotent Stem Cells. We have several ongoing projects aiming at producing large amount of genetically homogenous, genetically modified red blood cells that will be used as reagent red blood cells and for transfusion of allo-immunized sickle cell disease patients, and as carrier for replacement therapy of a variety of diseases including TTP and hemophilia A.

#### **GENE THERAPY:**

We are studying the biology of hematopoietic stem cells in the context of sickle cell disease and have developed a CRSPR-based methods to perform knock-in of mini-circles in human hematopoietic stem cells for gene therapy of hemoglobinopathies and other genetic disorders affecting erythroid cells.

**FOR MORE DETAILS:** <https://www.einstein.yu.edu/faculty/4981/eric-bouhassira/>

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## **Single Molecule Imaging of Transcription Factors Involved in Cancer, Hematopoiesis and Ageing**

Human tumorigenesis is a complicated process marked by a loss of the cell's ability to regulate critical cellular processes, such as transcription, RNA processing and translation, leading to uncontrollable cell growth. As our understanding of tumorigenesis becomes more sophisticated, a combinatorial approach is necessary to better understand the dynamic coordinated action of very large multi-subunit enzymes and protein complexes controlling these key cellular processes. Recent advances in single-molecule imaging provide an unprecedented spatiotemporal window into probing dynamic functional interactions between these heterogeneous large multi-subunit transcription assemblies and chromatin in real-time. In addition, high-resolution cryo-electron microscopy and genome-wide binding assays allow us to rapidly survey detailed functional interactions on physiologically relevant substrates with limited amounts of samples. Armed with these advanced *in vitro* and *in vivo* approaches, our lab seeks to gain a mechanistic understanding of how transcription complexes dynamically regulate expression of tumor suppression pathways and how these processes are altered in cancer.

Specifically we apply these tools to mechanistically dissect how the p53 tumor suppressor protein communicates with multiple transcription assemblies, such as chromatin remodeling factors (PBAF), core promoter recognition factors (TFIID), and RNA Polymerase II, to circumnavigate the repressive effects of chromatin on transcription. We are also applying these same strategies to study how oncogenic mutants of p53 and chromatin-remodeling complexes mechanistically function to promote tumor formation. Our long-term goal is to determine the molecular origin of cancer and additional diseases related to hematopoietic dysfunction, eye development and ageing using our multi-disciplinary approach centered around advanced single molecule imaging, genome-wide studies, and structural biology.

### **Development of single molecule imaging systems to study how transcription factors alter chromatin structure and regulate transcriptional bursting**

To understand how proteins engage chromatin at high temporal and spatial resolution, our group has established numerous *in vitro* and *in vivo* systems utilizing high-resolution co-localization, single molecule FRET, and dynamic live cell imaging. Strikingly, our live cell imaging studies find that transcription factors and chromatin remodelers, including p53, RNA Polymerase II and PBAF, dynamically cycle on and off the genome in spatial hubs of activity on the timescale of seconds to minutes. The chromatin structure of underlying target sequences and enzymatic activities associated with the transcription factors/chromatin remodelers dictate their dynamic cycling and binding kinetics. Oncogenic mutations in transcription factors can also affect the binding kinetics of associated proteins that are being co-loaded onto the genome. In addition, we find that single molecule kinetic binding profiles of transcription factors are associated with different cellular states during development. Furthermore, we are also adapting our imaging systems to directly assess the enzymatic activity of chromatin remodelers and enzymes that translocate along the genome.

In collaboration with Rob Singer's lab at Einstein, we have also developed a live-cell multicolor single molecule imaging system to examine how our transcription factors dynamically regulate transcriptional bursting of tumor suppression genes. Using these imaging systems, we find that patterns of transcription factor binding and transcriptional bursting display memory effects to fine-tune expression of genes. Future work will be to develop this high resolution imaging in live mice and live-cell image based drug screening platforms that rapidly determine combinatorial effects of epigenetic inhibitors in different cell types and diseased cells.

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## **Tumor MicroEnvironment of Metastasis**

### **Areas of Research:**

Cell biology and biophysics, cancer biology, and the integration and validation of clinical imaging platforms with high resolution intravital multiphoton microscopy. Development of multiphoton imaging technology to identify tumor cell dissemination mechanisms in both primary and secondary tumor sites during metastatic progression. Development of new optical microscope technology in the Gruss Lipper Biophotonics Center.

### **Professional Interests:**

John Condeelis' research interests are in optical physics, cell biology and biophysics, cancer biology and mouse models of cancer. He and his collaborators developed the multiphoton imaging technology and animal models used to identify invasion and intravasation micro-environments in mammary tumors. Integration of intravital multiphoton imaging with computational /systems analysis of living breast tumors identified the dominant tumor cell phenotypes contributing to invasion and dissemination during metastasis. This led to the discovery and verification of the paracrine interaction between tumor cells and macrophages in vivo, the role of macrophages in the migration of tumor cells during HGF-dependent tumor cell streaming to blood vessels and the mechanism of tumor cell dissemination from primary tumors via TMEM (Tumor MicroEnvironment of Metastasis) doorways to distant metastatic sites. Based on these results, cell collection techniques, including the in vivo invasion assay were developed for the collection of migrating and disseminating macrophages and tumor cells. This led to the discovery of the mouse and human invasion signatures, and the TMEM doorway, MenaCalc and MenaINV markers for assessing risk of metastasis and prediction of response in breast cancer patients to both chemotherapy, and receptor tyrosine kinase and tyrosine kinase inhibitors used to suppress metastasis.

John Condeelis has devised optical microscopes for uncaging, biosensor detection and multiphoton imaging for these studies and has used novel caged- enzymes and biosensors to test, in vivo, the predictions of the invasion signatures regarding the mechanisms of tumor cell dissemination and metastasis. He is one of the founding co-directors of the Integrated Imaging Program dedicated to the integration and validation of clinical imaging platforms, including digital pathology, with high resolution optical imaging in the Gruss Lipper Biophotonics Center. He has authored more than 350 scientific papers on various aspects of his research.

**For a complete list of publications please visit <https://www.einsteinmed.edu/labs/john-condeelis/publications/>**

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## **Genomic Instability and Cancer in Murine Models**

The maintenance of genomic integrity in all organisms requires multiple DNA repair pathways that are involved in the processes of DNA replication, repair and recombination. Perturbations in these pathways can lead to increased mutation rates or chromosomal rearrangements that ultimately result in cancer. DNA mismatch repair (MMR) is one of the repair systems that mammalian cells employ to maintain the integrity of its genetic information by correcting mutations that occur during erroneous replication. Mutations in MMR genes are linked to one of the most prevalent human cancer syndromes, Lynch syndrome and a significant number of sporadic colorectal cancers. At the molecular level tumors that develop in these patients display increased genomic mutation rates as indicated by increased mutations at microsatellite repeat sequences (termed microsatellite instability, MSI). MMR in eukaryotes is complex and involves several homologs of the bacterial MutS and MutL proteins. In mammals, the initiation of the repair process requires two complexes formed by three different MutS homologs (MSH): A complex between MSH2-MSH6 for the recognition of single base mismatches and a complex between MSH2-MSH3 for the recognition of insertion/deletions. The repair reaction also requires a complex between the two MutL homologs MLH1 and PMS2 that interacts with the MSH complexes to activate subsequent repair events which include the excision of the mismatch carrying DNA strand and its re-synthesis. In addition to correcting DNA mismatches, the MMR system mediates an apoptotic response to DNA damage and both of these functions are thought to be important for genome maintenance and tumor suppression. We have generated gene targeted mouse lines with inactivating mutations in all the different MutS and MutL homologs, and also in genes that function in the later MMR steps to study their roles in genome maintenance and tumor suppression. In addition, we have generated knock-in mouse lines with missense mutations and conditional knockout mouse lines that inactivate specific MMR functions and/or model mutations found in humans. Our studies indicate that specific MMR functions play distinct roles in maintaining genome stability and that defects in these functions have important consequences for tumorigenesis. These studies have also revealed that MMR proteins play essential roles in class switch recombination and somatic hypermutation during antibody maturation and the control of meiotic recombination in mammals. We are currently studying the functions of MMR in intestinal stem cells (ISCs) and cancer stem cells (CSCs) in preclinical mouse models and how loss of MMR in stem cells affects tumorigenesis and the response of tumors to novel anticancer treatments including immune therapeutic approaches.

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## **BIOCHEMISTRY AND GENETICS OF CHROMATIN TRANSITIONS IN *DROSOPHILA***

Hundreds of millions of base pairs of nuclear DNA are packed into chromosomes. Chromatin, the nucleoprotein filament of a chromosome, has many organization levels. It is the natural state of DNA in the nucleus and the native substrate for DNA-directed reactions, such as DNA replication, recombination, repair and transcription. The assembly of chromatin and dynamic conversion between its different forms are critical steps in the maintenance and regulation of the eukaryotic genome. The goal of our research is to understand how chromosomes are assembled and how this process regulates the structure and activity of eukaryotic chromosomes. The crucial first step in this direction is a systematic study of factors that mediate this process. To this end, we use biochemical approaches to analyze their contributions to chromatin assembly. We also dissect their function *in vivo* by methods of *Drosophila* genetics. More globally, we are trying to elucidate the roles nuclear enzymes in the hierarchical organization of the chromosome.

### **1. Nucleosome assembly mechanisms and biological functions of chromatin assembly factors**

*In vivo*, canonical nucleosomes are assembled by two families of proteins, core histone chaperones, such as NAP-1, and ATP-dependent motor factors, such as ACF (ATP-utilizing Chromatin assembly Factor). ACF consists of a SNF2-like ATPase ISWI and a polypeptide termed Acf1. We study ATP-dependent chromatin assembly *in vitro* to elucidate molecular events that take place during the formation of nucleosomes.

In addition to ACF, two other ISWI-containing complexes, ToRC (comprising Tou, ISWI and CtBP) and RSF (Rsf1 and ISWI), mediate similar reactions and can functionally substitute ACF *in vivo*. Our genetic and cytological analyses implicate the network of ATP-dependent, ISWI-containing chromatin assembly factors in diverse, partially redundant pathways of regulation of chromatin structure and activity. SNF2-like protein CHD1 is another ATP-dependent nucleosome assembly factor. It is required for replication-independent deposition of histones into chromatin *in vivo*. Specifically, CHD1 is essential during early embryonic development for deposition of replacement histone H3.3 into paternal chromatin.

### **2. Mass spectrometry tools for the analyses of chromatin structure and stable protein complexes**

We have developed a novel MRM-HR method to precisely quantify the relative content of H3.3 in chromatin from very limiting native sources. We demonstrated that *Drosophila* CHD1 is required for the assembly of H3.3-containing nucleosomes in adult fly brain, and its deletion results in severe physiological consequences, including metabolic mis-regulation and reduced lifespan.

The factors of nuclear DNA metabolism rarely act as individual polypeptides but, rather, within stable multi-subunit complexes. The existing methods to characterize native complexes suffer from several drawbacks. Tag-affinity purification of ectopically expressed subunits is prone to an unacceptably high rate of false-positive IDs. On the other hand, conventional chromatography to achieve apparent homogeneity is time-consuming and can only be applied to abundant proteins. We have devised, implemented and validated a highly innovative, streamlined method termed MS-Enabled Rapid protein Complex Identification (MERCI) to rapidly identify stable native complexes. It relies on partial enrichment of a target protein in sequential FPLC steps, quantitative shotgun proteomics of chromatographic fractions and correlation analyses. MERCI allows rapid identification of scarce complexes without a loss of the precision emblematic of the classical FPLC approach. It makes characterization of native complexes a routine laboratory technique and allows characterization of trace amounts of protein complexes with a virtually assured result.

### **3. Higher-order chromatin forms**

To reconstitute higher-order chromatin structures, we supplement the *in vitro* assembly system with modified core histones, histone variants, linker histone (H1) and heterochromatin proteins, such as *Drosophila* HP1a. Chromatin vectors can turn into useful tools in research and therapy. These studies will also eventually lead to the discovery of techniques to reconstitute functional metazoan chromosomes.

We examined phenotypes associated with the depletion of H1 by RNAi in flies. We discovered that H1 is the major component of heterochromatin and is required to establish its biochemical identity and functional properties. For instance, H1 recruits HMT Su(var)3-9, which mediates methylation of lysine 9 of histone H3

(H3K9), a signature heterochromatin-specific epigenetic mark. We have also demonstrated that H1 is essential for the faithful regulation of DNA endoreplication in *Drosophila* larval cells. H1 negatively regulates DNA replication in conjunction with a SNF2 ATPase SUUR that forms a native complex (SUMM4) with an insulator protein Mod(Mdg4).

A prevalent view of heterochromatic silencing is that its physical compaction results in steric exclusion of regulatory proteins, such as RNA polymerases. In collaboration with G. Karpen (LBNL), we have recently shown that the formation of heterochromatin domains is also mediated by liquid-liquid phase separation that gives rise to a non-membrane-bound nuclear compartment. We demonstrated that HP1a and H1 undergo demixing *in vitro* and nucleate into foci that display liquid properties during heterochromatin domain formation in early *Drosophila* embryos. We propose that biophysical properties associated with phase-separated systems are critical to understanding the behavior of heterochromatin and, potentially, other chromatin forms that regulate essential nuclear functions.

#### 4. Sperm chromatin assembly and remodeling

In sperm, DNA is compacted with cysteine-rich protamines and protamine-like sperm nuclear basic proteins (SNBPs) to form enzymatically static sperm “chromatin”. We have begun to analyze protein factors that mediate SNBP deposition during spermatogenesis and their removal from DNA after fertilization. It turns out that sperm chromatin assembly and remodeling is mediated by a group of factors that are similar to core histone chaperones.

Upon deposition on sperm DNA, protamines/SNBPs are extensively crosslinked via interchain disulfide bonds. After fertilization, the egg has to reverse the crosslinks for efficient eviction of SNBPs. This nuclear reaction is mediated by specific thioredoxin (TRX) and thioredoxin reductase (TRXR) molecules. Thus, we are investigating biological roles of the evolutionary conserved thioredoxin system in sperm chromatin metabolism and female fertility. A number of chemical compounds are known to specifically inhibit the function of TRX and TRXR proteins. We are studying their ability to suppress fertilization in the egg *in vivo* and testing their utility as novel, non-hormone agents for female contraception.

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### **MacroH2As, histone variants with diverse roles in gene expression and DNA damage responses**

– The macroH2A-type histone variants (which include macroH2A1.1, macroH2A1.2 and macroH2A2) have roles in tumor suppression, cellular senescence, activation and repression of transcription, promotion of DNA repair and suppression of the reprogramming of differentiated cells into stem cells. MacroH2As are typified by a histone H2A-like region fused by a flexible linker to a C-terminal macrodomain, a ligand-binding domains whose functions is modulated by binding to poly(ADP-ribose) produced by a family of poly(ADP-ribose) polymerases. MacroH2A1 regulates the expression of genes found within its large chromatin domains which can span hundreds of kilobases. Through changes in its expression and/or alterations in its genomic localization, disruption of macroH2A1's tumor suppressive functions are common in cancer; alterations of macroH2A transcription and splicing occur in a variety of cancers including those of lung, breast, colon, ovaries, endometrium, bladder, testicles, and melanocytes. Consistently, macroH2A1 loss in primary cells is sufficient to trigger an oncogenic gene expression profile. We are interested in many aspects of macroH2A biology. 1) How are macroH2As targeted to specific regions of the genome? 2) How does macroH2A1.1 in collaboration with PARPs regulate gene expression? 3) How does macroH2A1 regulate chromatin accessibility at enhancers? 4) How does macroH2A participate in DNA repair? 5) What regulates macroH2A1's alternative splicing?

**Chromatin dynamics during oncogene-induced senescence and cancer** – Oncogene-induced senescence (OIS) is an important tumor suppressive mechanism whereby a cell harboring an oncogenic mutation enters a stable proliferative arrest. At the same time the senescent cell secretes a host of inflammatory cytokines, chemokines and metalloprotease called the senescence-associated secretory phenotype (SASP), which serves to recruit immune cells to clear the senescent cells from tissues. The histone variant macroH2A1 plays a critical role in the transcriptional regulation of SASP genes during senescence. We are currently studying the mechanism by which macroH2A regulates the SASP response. We hypothesize that changes in macroH2A1 expression, seen in many cancers, allows these cells to bypass senescence and proceed on the pathway towards transformation.

**Interplay between transcriptional elongation rates and alternative splicing** – Alternative splicing is a crucial aspect of gene expression, allowing a gene to yield functionally distinct products, the abundance of which are regulated by cellular cues. Splicing dysregulation is central to several cancers and developmental diseases. Alternative splicing can be regulated through the recruitment of splicing factors which promote or repress distinct splicing events. Splicing largely occurs co-transcriptionally, and so, splicing outcomes are also affected by aspects of the transcription process and chromatin environment. The local elongation rate of RNA polymerase II is one aspect of transcription with important consequences on splicing outcomes. A barrier to progress in the field has been the lack of a high-throughput assay to measure splicing rates in mammalian cells. To address this, we have developed SKaTER-seq (Splicing Kinetics and Transcript Elongation Rates through sequencing). With this assay, we are exploring a myriad of factors that regulate splicing, including elongation rate, gene architecture, binding sites for RNA binding factors, chromatin structure and histone modifications. With this powerful approach we will determine the underlying causes of splicing alterations in disease.

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## **Kinase signaling pathways in adult blood development and blood cancers**

My lab studies the signal transduction pathways that affect the early fate decisions of adult hematopoietic stem cells (HSCs) as they progress from an undifferentiated multipotent state to the generation of differentiated blood cells. When these early fate decisions go awry, this can lead to the formation of leukemic stem cells, which can initiate leukemia and contribute to relapse after treatment. We use a combination of genetically modified mice, cell lines, and patient samples to explore the mechanisms by which signaling pathways maintain both normal and leukemic hematopoiesis.

### **Roles of the PI3 kinase isoforms in adult blood development**

PI3 kinase (PI3K) is a lipid kinase that is important for the regulation of metabolism, the cell cycle, apoptosis, protein synthesis, and autophagy. In hematopoietic cells, there are four isoforms of the catalytic subunit of PI3K, each encoded by a separate gene. Emerging evidence suggests that these isoforms have unique functions in normal and cancer cells, but may substitute for each other in some contexts. We have generated a series of mouse knockout models that allow us to study the roles of each of these isoforms individually in adult hematopoiesis. For example, we have found that the p110alpha isoform is most important for red cell development, but is not required in normal blood stem cells. We have now also generated compound knockout mice to determine the redundant roles of the PI3K isoforms in blood development. We have found that deletion of multiple PI3K isoforms impacts normal HSC function, including self-renewal, proliferation after infection or chemotherapy treatment, and differentiation along different blood lineages.

### **Roles of kinase of signaling pathways in blood malignancies**

Acute myeloid leukemia (AML) is a genetically diverse disease, but activation of the PI3K pathway has been reported in up to 80% of cases. We are using several different mouse models of AML to examine the roles of individual PI3K isoforms in leukemic stem cells (LSCs), which is the cell population in AML patients that has self-renewal activity, and has been implicated in relapse. In particular, we are exploring how PI3K cooperates with epigenetic regulators in LSCs, and how PI3K in LSCs can promote evasion of the immune system. We are also studying the roles of PI3K and other signaling molecules in the normal and leukemic bone marrow microenvironment.

In addition, we recently discovered that RON kinase can be a new potential therapeutic target in a different blood cancer called myeloproliferative neoplasms (MPN). Future work will focus on understanding the mechanisms of RON signaling and regulation in MPN cells, and on strategies for therapeutic targeting of RON in this disease.

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**Key Words:** *mammary stem/progenitor cells, breast cancer stem cells, drug resistance, metastasis*

My lab is interested in two interlocking areas of stem cell biology and cancer biology: the molecular pathways that regulate the normal stem-cell fate in the mammary gland, and the role of stem cell fate/pathway dysregulation in breast cancer pathogenesis.

### **Role of mammary stem cells in cancer initiation**

We have developed sensitive and specific in vitro and in vivo mammary stem cell assays. Using these assays, we have identified novel unipotent stem cells that are responsible for the development of distinct mammary epithelial cell lineages. We are investigating whether these unipotent stem cells are the cell-of-origin for different breast cancer subtypes. In addition, we are elucidating the key cell fate determinants of these distinct stem cells, and investigating oncogenic mechanisms causing the dysregulation of stem cell fates during tumorigenesis.

### **Function of stem-cell pathways in breast cancer progression and metastasis**

Emerging evidence suggests that normal stem-cell pathways often get activated aberrantly in cancers and contribute to aggressive cancer behaviors. Identification of key normal stem cell regulators provides us a framework to understand how breast cancer stem cells are regulated. We are particularly interested in understanding the role of stem-cell factors in regulating metastatic colonization, a rate-limiting step of the metastatic cascade that involves cancer stem cells. In addition, we are interested in how cancer stem cells are regulated by the tumor microenvironment.

### **Selected Publications**

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**Key Words:** *Hematopoietic stem cell, Leukemia, Myelodysplastic syndrome, Sickle cell disease*

The central research goal of the Ito Lab is the expansion of our understanding of the regulatory pathways **controlling the equilibrium of healthy and malignant hematopoietic stem cells**. At the core of our work is the process of stem cell division, and the resulting balance between **self-renewal or differentiation**, which directly impacts tissue homeostasis. We are also devoting increased attention to targeting **cellular metabolism** and/or **mitochondrial physiology** as a therapeutic strategy, and have cut a path along the leading edge of research into the role of **epigenetic-microRNA crosstalk**, including physiologically relevant Ten-eleven translocation, in the pathogenesis of **myelodysplastic syndrome**. We believe our expertise in stem cell biology, hematology, and the bone marrow microenvironment, combined with our development of **single-cell approaches** and **live imaging to tracking stem cell fate in vivo** and **leukocyte behavior in sickle cell disease** animal models, will together facilitate a major contribution to the improvement of transplantation efficiency and the development of new therapies and treatments, and potentially even cures, for many forms of **hematologic pathology**.

**Selected Original research and Theoretical treatises;**

1. Morganti C et al., NPM1 ablation induces HSC aging and inflammation to develop myelodysplastic syndrome exacerbated by p53 loss. **EMBO Rep.** 2022 May 4;23(5):e54262.
2. Ma L et al., Tet-mediated DNA demethylation regulates specification of hematopoietic stem and progenitor cells during mammalian embryogenesis. **Sci Advances.** 8, eabm3470 (2022). doi: 10.1126/sciadv.abm3470.
3. Schönberger K et al., Multilayer omics analysis reveals a non-classical retinoic acid signaling axis that regulates hematopoietic stem cell identity. **Cell Stem Cell.** 2022 Jan 6;29(1):131-148.e10.
4. Wu HC et al., Actinomycin D targets NPM1c-primed mitochondria to restore PML-driven senescence in AML therapy. **Cancer Discovery.** 2021 Jul 23.
5. Wu JW et al., Intravital fluorescence microscopy with negative contrast. **PLoS One.** 2021 Aug 5;16(8):e0255204.
6. Morganti C, Bonora M, Ito K, **Ito K.** Electron transport chain complex II sustains high mitochondrial membrane potential in hematopoietic stem and progenitor cells. **Stem Cell Res.** 2019 Oct;40:101573.
7. Ito K\*, Lee J\* et al., Non-catalytic roles of Tet2 are essential to regulate hematopoietic stem and progenitor cell homeostasis. **Cell Rep.** 2019 Sep 3;28(10):2480-2490.e4.
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10. **Ito K** et al. PML targeting eradicates quiescent leukaemia-initiating cells. **Nature.** 2008 Jun 19;453:1072-8.
11. **Ito K\***, Hirao A\* et al. Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. **Nature.** 2004 Oct 21;431(7011):997-1002.

**Selected Invited publications;**

1. Torres LS et al., Recent advances in “Sickle and Niche” research. **Stem Cell Reports.** in press
2. Morganti C et al., Metabolic Regulation of Hematopoietic Stem Cells. **HemaSphere.** July 2022 (7) p e740.
3. Ito K et al., Leukemia stem cells as a potential target to achieve therapy-free remission in chronic myeloid leukemia. **Cancers.** 2021 Nov 20;13(22):5822.
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## **Molecular Mechanisms of Virus Entry and Exit.**

For more information, please see our lab homepage:

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All enveloped viruses use the essential steps of membrane fusion to enter a host cell, and membrane budding to exit. Molecular information on the entry and exit processes is critical to understanding the lifecycle of enveloped viruses and how they exploit the host cell machinery, and as an important model for cellular membrane fusion and budding reactions.

Our research focuses on the molecular mechanisms of virus entry and exit using alphaviruses and the closely related virus rubella virus, and flaviviruses such as dengue virus. The flaviviruses and alphaviruses include many important human pathogens such as dengue, Zika, and chikungunya viruses, which cause millions of human infections each year. There are no vaccines or antiviral therapies for most of these viruses, and new strategies are urgently needed.

Alphaviruses, rubella virus and flaviviruses enter cells by endocytic uptake and then fuse their membrane with the endosome membrane in a reaction triggered by the low pH of the endocytic vesicle. The membrane fusion proteins of these viruses are structurally related proteins and refold during fusion to a homotrimer conformation that mediates virus fusion and infection. Recent studies have also shown that structurally similar proteins are expressed in plants and in many animals, where they mediate cell-cell fusion of gametes and during development.

Many important questions on the molecular mechanism of membrane fusion remain for both viruses and cells. Little is known about the mechanism and structural features of fusion protein insertion into the target-membrane. We are also investigating the pH-dependent control mechanisms for the rubella virus fusion reaction.

During alphavirus and flavivirus biogenesis, a companion protein forms a closely-associated dimer with the fusion protein, and protects it from low pH and premature fusion during exocytic transport. This companion protein must then dissociate to permit virus fusion. The pH protection mechanisms for many other viruses are unknown, and we are using rubella virus as a system to define novel mechanisms of pH protection.

Alphaviruses exit by budding through the plasma membrane of the infected host cell. Little is known about alphavirus assembly and budding, although it is clear that these processes are highly regulated to produce organized virus particles of high specific infectivity. How does this happen and what are the roles of cellular and viral factors? We seek to determine how the internal viral RNA-capsid core is assembled, how the virus excludes host RNAs, and how nucleocapsid assembly can be inhibited by small molecules. We are using a novel capsid protein retrieval strategy to identify and characterize host factors involved in alphavirus nucleocapsid assembly. We have developed fluorescently tagged alphaviruses to follow virus assembly and budding in real time in infected cells. We are investigating how alphaviruses spread from cell to cell, a process that protects the virus from antibody neutralization. The cell plasma membrane and cytoskeletal network are dramatically remodeled during budding and we are defining the mechanisms and signaling pathways that mediate remodeling.

Our lab uses a wide variety of approaches including molecular biology, virus genetics, protein biochemistry, live cell imaging, cell biology, and structural biology.

Potential research projects include: investigation of specific molecules involved in cell-to-cell virus transmission, use of fluorescently tagged viruses to follow steps in virus assembly and budding, characterization of the role of cellular factors in virus assembly and exit, use of virus mutants to characterize specific steps in fusion and pH protection.

#### **Complete List of Published Work:**

<https://www.ncbi.nlm.nih.gov/sites/myncbi/margaret.kielian.1/bibliography/45469240/public/?sort=date&direction=descending>

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## **Cell Death: Fundamental Mechanisms and Roles in Human Disease**

The most basic decision that any cell makes is to grow, differentiate, or die. Our laboratory studies fundamental mechanisms of cell death and the roles of cell death in normal biology and human disease. Twelve cell death programs have been recognized to date. Little is understood about how they interconnect mechanistically or integrate functionally to bring about cell death. *The overarching goal of our work is to create a more unified model of cell death that integrates seemingly “individual” cell death programs.* Our hypothesis is that “individual” cell death programs, which often undergo activation in the same cell, are components of an integrated cell death response that has arisen over evolution.

To gain traction in understanding this integration, we have focused on two cell death programs, mitochondrial-dependent apoptosis and mitochondrial-dependent necrosis. The rationale for choosing these programs is that the sentinel events in each take place within microns of each other at the outer and inner mitochondrial membranes respectively. In addition, both of these programs play important roles in the pathogenesis of the major human diseases.

We have adopted two strategies to understand connections between mitochondrial-dependent apoptosis and necrosis. The first is a candidate approach to identify mediators which are common to both programs and, therefore, may function as “decision points”. These studies have revealed that several canonical regulators of apoptosis, including BAX, ARC, and CASPASE-9, mediate necrosis through distinct mechanisms (Mol Cell, 2004; PNAS, 2007; JBC, 2007; PNAS, 2012; Cell Death Differ, 2014). A second approach has involved genetic screens to better define mitochondrial-dependent necrosis signaling, which remains poorly understood. These have included genome wide CRISPR-based functional screens and protein interaction screens using as bait proteins with known roles in this necrosis pathway. The results of these screens are currently the basis of several ongoing projects involving cell death, metabolism, and other aspects of mitochondrial function.

While the primary focus of the lab is basic mechanisms, we are also interested in translational implications. While some of our work has involved cell and mouse models of cancer (Cell Death Differ, 2005; JBC, 2010; Cancer Res, 2011, PLOS One, 2015) and type 2 diabetes (Diabetes, 2013; Sci Rep, 2017; Dev Cell, 2021), our primary translational focus is heart disease, the major cause of mortality worldwide. A number of years ago, our lab provided the initial evidence that regulated forms of cell death play critical roles in the pathogenesis of myocardial infarction (“heart attack”) (Circulation, 2000; JMCC, 2000; AJP, 2003) and heart failure (JCI, 2003). More recently, the lab contributed to the development of small molecule and peptide drug prototypes that inhibit cell death and may provide the basis for new therapies for heart diseases and other conditions (Nature, 2016; Science, 2018; Nat Chem Biol, 2019; Nat Cancer, 2020). We continue to use the *in vivo* myocardial infarction model in our current work aimed at unifying cell death mechanisms because at least 6 cell death programs are activated in this syndrome.

Projects currently in progress:

1. Mechanism of CASPASE-9-mediated necrosis
2. Functions of BCL-2 proteins at the outer and inner mitochondrial membranes
3. Regulation of ferroptosis by OPA1
4. Contributions of necroptosis and ferroptosis to myocardial infarction
5. Mitochondrial-ER/SR interactions in regulating cell death and metabolism
6. Role of the mitochondrial ATP synthase in bioenergetics and cell death
7. Identification of the mitochondrial permeability transition pore
8. Determinants of the senescence versus cell death “decision” in aging tissues

Link to publications:

<https://pubmed.ncbi.nlm.nih.gov/?term=kitsis+rn&sort=date>

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### **Gene regulatory mechanisms that mediate lung cancer evolution**

Lung adenocarcinoma (LUAD) is the most common subtype of non-small cell lung cancer and remains a leading cause of cancer-related death. Next-generation sequencing studies have identified a number of recurrent genetic drivers (such as mutations in *KRAS* and *TP53*); however, this somatic variation does not fully explain key features of cancer evolution including progression toward metastasis and intratumoral heterogeneity. Our group aims to understand how non-genetic mechanisms contribute to lung cancer initiation and progression through the lens of chromatin biology.

In genetically engineered mouse modeling studies, cell-type specific transformation of alveolar type II (AT2) cells with *Kras*<sup>G12D</sup> and loss of *Tp53* lead to a natural cancer evolution process that recapitulates phenotypic hallmarks of human disease. Using this model, our recent work employed single-cell epigenomics to study dysregulation of chromatin state across lung cancer evolution. We identified a continuum of heterogeneous chromatin states with reproducible epigenomic features across individual lung tumors, suggesting conserved routes of cancer progression. Using both functional and computational approaches, we annotated transcription factor (TF) regulators and downstream gene programs associated with these diverse chromatin profiles. These discernable gene regulatory modules were linked to certain aspects of cancer progression including loss of cellular identity, inflammation, and metastasis. Our work also identified the emergence of a pre-metastatic gene regulatory program that arises in a rare cell population in primary tumors and primes cells for metastatic seeding. We identified RUNX2 as an important regulator of this cell state and are developing new models to study the role of RUNX TF biology in lung cancer progression.

Cellular identity is dictated by chromatin structure, which is maintained by a complex network of *cis*- (e.g., enhancer, promoters, and insulators), and *trans*- (e.g., chromatin modifiers, TFs, and adaptors) factors. Our previous work found that early stages of LUAD progression are associated with the loss of AT2 identity and the acquisition of epigenomic states similar to developmentally related cell types. We seek to further understand how lung cancer initiation ultimately leads to decoupling from an AT2 cell identity and increased epigenomic plasticity. In addition, we are interested in investigating how disruption of the tumor microenvironment influences LUAD cell state. To address these questions, we pair lung cancer modeling with epigenomic technologies (including single-cell ATAC-sequencing, single-cell multiomic analysis, and spatial approaches) to dissect mechanisms important for the maintenance and persistence of dysregulated cell states in lung cancer. We flexibly utilize genetically engineered mouse models, alveolar human and murine organoids, and cell-based assays to perform functional studies. We continue to expand our technological and modeling toolkit to study early and late stages of lung cancer progression with the overarching goal of better understanding and targeting lung cancer progression.

#### **Research directions in the lab:**

1. Perturbation of candidate chromatin regulators associated with aberrant cell states in lung cancer
2. Interrogation of mechanisms that drive epigenomic plasticity and cell identity shifts in LUAD
3. Investigation of the role of the tumor microenvironment and loss of tissue homeostasis in shaping cell state in lung cancer cells
4. Utilization of varied single-cell epigenomic technologies to decipher gene regulatory networks in normal lung and lung cancer cells

### **Selected Publications:**

\*Co-first; ^Co-corresponding authors

LaFave LM<sup>^</sup>, Savage RE, Buenrostro JD<sup>^</sup>. Single-cell epigenomic reveals mechanisms of cancer progression. *Ann. Review of Cancer Biology*. Apr 6:167-185.

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## **Mechanisms that regulate maintenance of niches for hematopoietic stem cells in aging and cancer.**

Life-long production of blood depends on the ability of hematopoietic stem cells (HSCs) to self-renew, differentiate, and form all blood cell lineages. HSCs rely upon a tight interaction with their microenvironment in the bone marrow (also termed the “niche”) to preserve quiescence and maintain normal blood output. These interactions are based upon membrane-bound, locally secreted, and/or long-range signals produced by a complex network of blood vessels, sympathetic nerve fibers, mesenchymal stem cells (MSCs), stromal cells, and hematopoietic cells. Aging of the hematopoietic system is associated with an age-dependent decline in HSC function leading to myeloid cell expansion and a reduction in lymphoid output that contribute to the development of myeloid malignancies. Many advances have been made toward deciphering intrinsic mechanisms that control HSC aging. However, the dependence of HSC dysfunction on the aging microenvironment remains underexplored.

Our interest is to understand mechanisms that control niche aging and contribute to the remodeling of niches that support myeloid malignancy and leukemic stem cells (LSCs). We use rigorous genetic models that target different niche constituents combined with innovative 3D imaging technology to detect endogenous HSC distribution in their native microenvironment. These approaches will provide novel insights into the molecular mechanisms underlying age-dependent stem cell dysfunction and identify factors that can be targeted to develop novel therapies to rejuvenate stem cell niches.

### **Primary interests of the lab:**

**1. Sympathetic nerves and adrenergic signals as regulators of niche homeostasis.** Our previous work established that signals from the sympathetic nervous system (SNS) control hematopoietic aging and highlight niche-derived factors as critical regulators of HSC longevity, rejuvenation of which can directly benefit aged stem cells (*Maryanovich et al., Nature Medicine 2018*). Future efforts are oriented toward identifying targets downstream of the SNS that control niche and HSC aging.

**2. Reactive oxygen species (ROS) role in remodeling niches to support myeloid malignancies and leukemic stem cells (LSCs).** Myeloid malignancies elicit alterations to the HSC niche that promote oncogenic expansion and eradication of healthy stem cells. The SNS was shown to be protective against myeloproliferation, as sympathetic denervation or deletion of  $\beta$ -adrenoreceptors accelerated the development of myeloproliferative disease (*Hanoun et al., Cell Stem Cell 2014*). Our work has shown that SNS signals control homeostasis of niche-derived ROS and that leukemic progression favors high oxidative stress, suggesting that SNS signals may mitigate ROS levels in the BM microenvironment to protect HSC niches during leukemogenesis.

**3. HSC niche metabolism and its role during aging and leukemogenesis.** Our goal is to determine how aging and leukemogenesis affect the metabolic activity of niche constituents and perturb their ability to support hematopoiesis. We will investigate the role of the SNS in controlling



niche cell metabolism and whether disruption of metabolic activity in the niche can deteriorate both its structure and function to facilitate hematopoietic aging and precondition niches to support leukemic infiltration. We will focus on glucose metabolism, fatty acid metabolism, and mitochondrial oxidative phosphorylation, pathways shown to be critical for stem cell maintenance.

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Pinho S, Wei Q, **Maryanovich M**, Zhang D, Pierce H, Nakahara F, Di Staulo A, Bartholdy B, Xu J, Borger D, Verma A and Frenette PS. VCAM1 confers innate immune tolerance on haematopoietic and leukaemic stem cells. **Nature Cell Biology**. 2022

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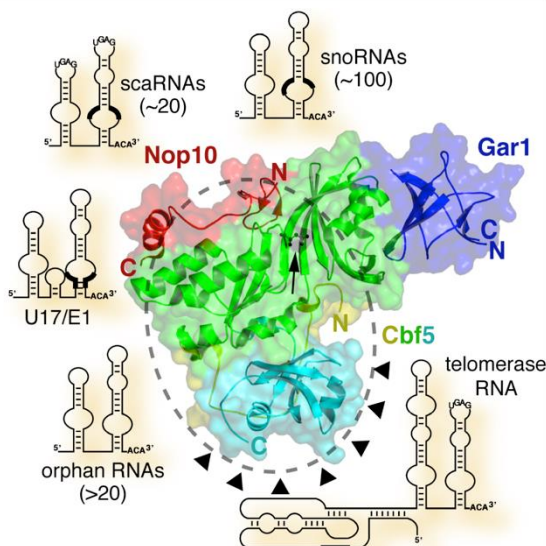
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## RIBONUCLEOPROTEINS IN CANCER AND HUMAN REPRODUCTION

Our group is studying the mechanism and regulation of nucleolar ribonucleoprotein biogenesis in relation to genetic disease, cancer, and human reproduction. Presently we are pursuing two main areas of research:

First, we are analyzing the biogenesis and function of small nucleolar ribonucleoproteins (snoRNPs) of the H/ACA class and how minor deviations from their natural assembly pathway can lead to cancer and bone marrow failure. Human H/ACA ribonucleoproteins are important for many basic cellular processes including protein synthesis, pre-mRNA splicing, and genome integrity. The different functional classes of H/ACA RNPs isomerize some 130 uridines to pseudouridines in ribosomal (r) and spliceosomal small nuclear (sn) RNAs, process rRNA, stabilize telomerase RNA, yield microRNAs, and harbor yet to be determined roles. Each of these functions is specified by one of over 500 H/ACA RNAs, each of which associates with the same four core proteins to form an H/ACA RNP. The central core protein, NAP57 (aka dyskerin or in yeast Cbf5p), is mutated in the predominant X-linked form of the inherited bone marrow failure syndrome dyskeratosis congenita (DC). NAP57 is an oncogenic protein, whereas the H/ACA RNP-specific assembly factor SHQ1 is a tumor suppressor of prostate and other cancers. We identify how disease causing and oncogenic point mutations in these proteins perturb H/ACA RNP assembly and how it can lead to an imbalance in cellular protein expression and genome instability. Among other cell biological approaches, we are employing CRISPR/Cas9 and RNAseq technology to get to the bottom of this. We are further collaborating with structural biologists and clinical scientists.



Meier, TIBS 2006

Second, we investigate the function of nucleolar channel systems (NCSs) in the cell and in human reproduction. During the height of receptivity of each menstrual cycle, NCSs transiently develop in the nuclei of endometrial epithelial cells (EECs). They are implicated in the preparation of the endometrium for uterine attachment of the fertilized egg. Although the molecular mechanisms of embryo implantation in humans are poorly understood, NCSs remain unexplored as candidate markers or potential prerequisites for implantation. This can be attributed to the fact that, despite their discovery over 50 years ago, until recently identification of NCSs was limited to electron microscopy. We identified molecular markers of NCSs, which finally affords simple and quantitative detection of these organelles at the light microscopic level. We are now exploiting our discovery to understand the cellular biology of NCSs and their regulation and function in uterine biology. To boost success rates of embryo

transfer during in vitro fertilization cycles, we apply our technology for timing endometrial receptivity before embryo transfer by NCS detection in uterine secretions.

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## Genetics of hematopoietic stem cell (HSC) clonal dynamics and leukemia predisposition

Every day, each one of us produce hundreds of billions of specialized blood cells through a process of hematopoietic differentiation originating from a pool of long-lived hematopoietic stem cells (HSCs). As we age, some HSCs acquire mutations that causes them and their progeny to expand resulting in the formation of their own distinct “clones”. This condition known as clonal hematopoiesis is ubiquitous with advancing age affecting 10%-20% of population by the age of 70 years. Individuals with clonal hematopoiesis have an increased risk hematological malignancies such as leukemia and myelodysplastic syndromes. If we could identify and prevent such clones from expanding this can dramatically reduce risk for blood cancers. Despite its importance, we still don't understand why these clones emerge only in some individuals and why only a small subset of such carriers' eventually progresses to cancer?

Our laboratory leverages human genetic studies such as genome-wide association studies (GWAS) and studies on rare families to address these questions. Our goal is to identify and experimentally study germline risk variants that predispose individuals to clonal hematopoiesis and myeloid malignancies. Our recent work has uncovered a previously unappreciated mechanism by which inherited genetic variants influence clonal blood disorders by modulation of HSC function and self-renewal (Bao et al., *Nature* 2020; Bick et al., *Nature* 2020).

We routinely model genetic risk variants into primary human hematopoietic stem and progenitor cells using CRISPR/Cas9 approaches and study their impact on stem cell function and clonal expansion of mutant HSCs. We are developing engineered mouse models to study mechanisms of blood cancer predisposition. Since the majority of these genetic variants are in noncoding genomic regions, we are employing high throughput approaches that we have developed to systematically test their effects on transcriptional activity (Ulirsch et al., *Cell* 2016). Our studies will provide critical insights into how germline variants influence the emergence of clonal hematopoiesis and its progression to hematological malignancies.

## Research Directions

- 1) Mechanisms of germline predisposition to clonal hematopoiesis and leukemia.
- 2) Investigation of the role of interactions between germline and somatic variants in shaping hematopoietic stem cell clonal fitness.
- 3) Genome engineering of human normal, preleukemic and leukemic hematopoietic stem and progenitor cells using CRISPR/Cas9.
- 4) Application of multiplexed functional assays and genetic screens to prioritize germline genetic variants underlying clonal blood disorders.

## Selected Publications

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## RNA Processing – Mechanisms and Disease

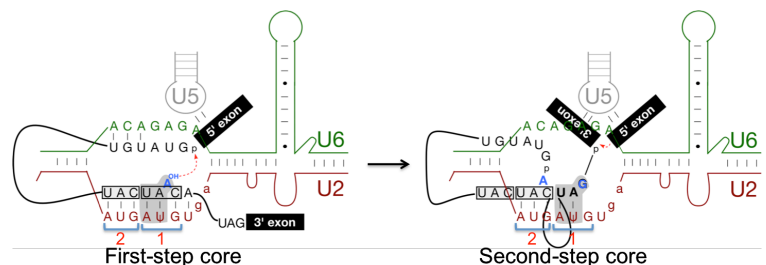
Intron removal, a defining feature of eukarya, is catalyzed by the spliceosome, a 50-60S complex composed of five snRNAs and >100 proteins. We investigate spliceosome function and spliceosomal mutations that cause disease.

**Do variant snRNAs make variant spliceosomes?** One ‘black hole’ in RNA biology is the identification of 50-300nt RNAs, missing in modern-day sequencing datasets. Vertebrate genomes reveal hundreds of snRNA gene loci; with few exceptions, only the most abundant canonical snRNAs have been investigated. We find that variant-snRNA expression changes greatly during development. How does this impact spliceosome function?

**Catalytic center interactions.** Base pairing of U2 snRNA to the intron branch site (BS) is essential for spliceosome assembly and first-step catalysis. We developed an orthogonal system where a second-copy U2 with grossly substituted BS-U2 base-pairs mediates splicing of a cognate reporter gene. This produces a non-essential second spliceosome that can be characterized in vivo. These properties allowed us to test whether the BS-U2 duplex exists at the time of first-step catalysis.

### What is the second-step catalytic core?

We are using our orthogonal systems to elucidate a 3'SS binding site within the second-step core, demonstrating that the first-step branch structure becomes partially unpaired from U2-GUAGUA, allowing the 3'SS to bind on U2 snRNA.

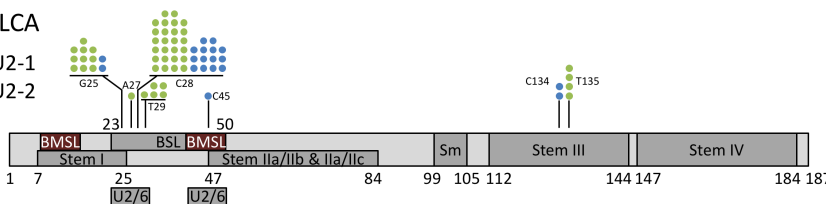


**Splicing and disease.** U2 snRNP protein SF3b1 and other U2 proteins are highly mutated in myelodysplastic syndromes and other cancers. However, there has been no information regarding U2 snRNA mutations. Our analysis of U2 variant loci allowed us to interrogate U2 RNA mutations in >1000 whole genome sequences (WGS) from the Cancer Genome Atlas (TCGA). We identified mutations in both U2-1 and U2-2 snRNAs, within the branch-recognition stem-loop (BSL), recurrent in bladder cancer (BLCA); we identified the same mutations in bladder cancer organoid lines (Lee et al., Cell 2018). The BSL contributes to recognition of the branch site as well as 3'SS. Genetic screens in yeast for improved splicing

of non-consensus 3'SS yield the same BSL mutation as found in bladder cancer. We used CRISPR-Cas9 to insert U2 mutations in human cell lines and are investigating the molecular consequences of these U2 mutations and the mechanism of resultant splicing changes.

BLCA

● U2-1  
● U2-2



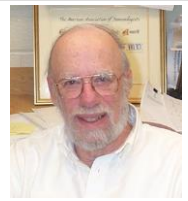
BSL = branch recognition stem-loop

BMSL = branch helix-mimicking stem-loop: Wojtek Galej, Science 2022



## Recent Publications (selected)

- Maron, M.I., Casill, A.D., Gupta, V., Roth, J.S., Sidoli, S., Query, C.C., Gamble, M.J., and Shechter, D. (2022). Type I and II PRMTs inversely regulate post-transcriptional intron detention through Sm and CHTOP methylation. *Elife*: e72867. doi: 10.7554/eLife.72867. PMID34984976 PMC8765754
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Our laboratory is studying how antibody-forming cells respond to antigen by undergoing somatic hypermutation and class switch recombination so that they can produce higher affinity antibodies with more useful effector functions. The molecular and biochemical mechanisms of antibody variable region hypermutation and class switch recombination is being studied in mice that have mutations in various repair proteins in collaboration with Dr. Winfried Edelmann. In order to examine detailed molecular mechanisms, we are also studying how mutation is targeted to antibody genes and some oncogenes in human Burkitt's lymphoma cell lines which are undergoing variable region mutation in culture. These cell lines are being used to study the role of activation induced deaminase (AID), mismatch repair and error prone polymerases in the variable region hypermutation and isotype switching. The analysis of these events also involves the examination of how changes in the DNA sequence of antibody genes, chromatin structure and transcriptional elongation lead to the targeting and regulation of AID. In collaboration with Dr. Thomas MacCarthy at Stony Brook, the role and evolution of the antibody V region sequence is also analyzed computationally using data bases of the human antibody response. The highly mutagenic processes required to generate antibody diversity also leads to B cell lymphomas and we are trying to understand how AID contributes to human Chronic Lymphocytic Leukemia in collaboration with Dr. Nicholas Chiorazzi from the Feinstein Institute at the Northwell Health Center.

We are also the Hybridoma Facility that helps investigators throughout the institution to make their own monoclonal antibodies.

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- 12) Post-transformation IGHV-IGHD-IGHJ mutations in chronic lymphocytic leukemia B cells: Implications for mutational mechanisms and impact on clinical course. Davide Bagnara, Catherine Tang, Jennifer Brown, Siddha Kasar, Stacey Fernandes, Monica Colombo, Stefano Vergani, Andrea Mazzarello, Fabio Ghiotto, Silvia Bruno, Fortunato Morabito, Kanti R Rai, Jonathan E Kolitz, Jacqueline C Barrientos, Steven L Allen, Franco Fais, **Matthew D Scharff**, Thomas MacCarthy, Nicholas Chiorazzi; Front Oncol. 2021 May 25;11:640731. doi: 10.3389/fonc.2021.640731. eCollection 2021.
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**Our laboratory is a part of the Einstein Center for Human Embryonic Stem Cell Research and the Cancer Center.**

## **Molecular Analysis of DNA Replication and Repair in Alzheimer's Disease and at Cancer-Associated Sites in the Human Genome**

A major interest of our lab is the organization and regulation of the DNA replication program in mammalian cells and its effect on gene expression. One area we have been concentrating on the role of DNA replication in neuronal diseases such as Alzheimer's. Alzheimer's disease (AD) is a neuro-degenerative disease that has been studied for more than three decades. Surprisingly after all this effort, there is still no unequivocal treatment, and the cause is not known.

We are also focused on understanding the role of genomic instability at human chromosomal fragile sites in cancer and aging.

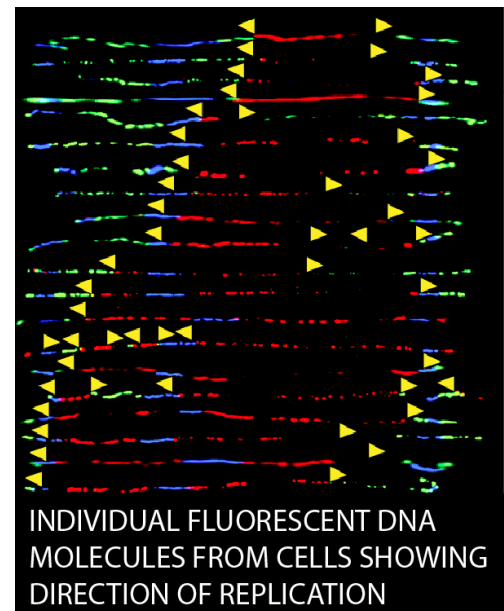
Genome instability occurs in the early development of many cancers and other diseases and is thought to be caused by replication fork stalling. This is clinically relevant since most current cancer therapies are based on radiation or chemotherapeutic agents that stall or block forks. Manipulating cellular responses to fork stalling may therefore prove crucial in improving effectiveness of current chemotherapies.

### **Long term interests:**

- Difficult to replicate regions accumulate secondary structures which, if not properly resolved, may block DNA replication fork movement and lead to genome instability. We are studying mechanisms to resolve these barriers for efficient and faithful DNA replication.
- The role of DNA replication of genes involved in neurological diseases such as Alzheimer's. We will examine critical genes in the brain that undergo mutations with aging.
- Genome protection by telomeres.
- Regulation and reprogramming of DNA replication of human embryonic stem (ES) cells and induced pluripotent stem cells (iPS).
- Mechanisms leading to breaks at sites that result in chromosomal rearrangements frequently detected in cancer cells.

### **Current projects include a wide range of interests:**

- The replication of two repeated sequence DNAs that are involved in Alzheimer's disease: alpha satellite DNA and the ribosomal DNA genes.
- The DNA2 nuclease/helicase can cleave G-quadruplex structures which are one of the most common non-B structures present in DNA. We study the effect on the deletion of DNA2 on DNA replication.
- Replication of the alpha satellite DNA at centromeres of specific human chromosomes. These include chromosome 21 and the Y chromosome. The Y chromosome is lost with aging, and we are studying the



replication of this chromosome to understand more about this loss. Chr 21 undergoes trisomy in Down syndrome and people with Down syndrome have a higher risk of getting Alzheimer's symptoms.

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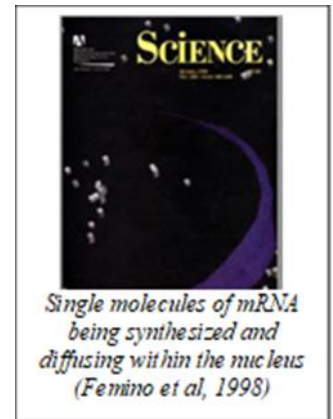


## **FOLLOWING THE TRAVELS OF RNA**

Our work is focused on the expression and travels of RNA within the cell: from the site of its birth to its ultimate biological destiny in the cytoplasm where it makes proteins in specific locations.

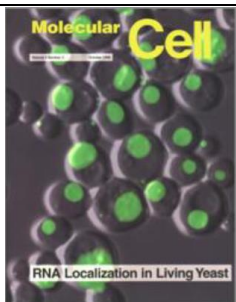
Our new technology, based on in situ hybridization allows us to visualize specific nucleic acid sequences within individual cells. Synthetic nucleic acid probes are labeled with fluorochromes. Subsequently these molecules are hybridized to the cell and detected using high resolution digital imaging microscopy.

We have developed imaging methodologies and algorithms capable of detecting a single RNA molecule within a cell. This enables the detection of specific nucleic acid molecules for comparison between normal or cancer cells. This method of molecular diagnosis is the clinical application of the technology. As an additional result of this approach, we have found specific RNA sequences located in particular cellular compartments. An example is the messenger RNA for beta-actin, which is located in the periphery of the cell where actin protein is needed for cell motility. These transcripts are not free to diffuse, and appear to be associated with a cellular matrix or skeleton from the moment of their synthesis through translation.



We are investigating how this spatial information is encoded within the gene and how the RNA transcript is processed within the nucleus and then transported to its correct compartment in the cytoplasm, resulting in asymmetric protein distribution.

RNA localization also occurs in yeast. During budding, a nuclear factor represses mating type switching asymmetrically, only in the daughter cell. This is because the factor is synthesized only in the bud because the mRNA was transported there by a motor, myosin. This discovery has provided a model by which to investigate the mechanisms responsible for moving RNA within the cell. For example, we have constructed genetically altered yeast and vertebrate cells in order to elucidate the sequences responsible for mRNA localization. A reporter gene can be "delivered" to a variety of cellular compartments by using specific sequences, or "zipcodes" from the mRNAs found in those compartments. These "zipcodes" consist of short sequences in the 3' untranslated region of the mRNA.



*Detection of mRNA movement in living yeast (Bertrand et al, 1998)*

Recently we have developed technology that allows us to image RNA movement in living cells and tissues and characterize how the motors connect with and drive the RNA. Recent developments have allowed us to visualize transcription and RNA life cycle from birth to death in transgenic mice, including translation of single mRNAs. We have labelled a number of genes in mice that are important in neuronal function and can image their mRNAs in cultured neurons and in brain tissue. The imaging reveals a complicated pathway for the mRNAs to arrive at sites of synaptic contact and translate in order to consolidate proteins into local "hotspots". This represents the molecular basis of memory formation.



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Chicurel ME, Singer RH, Meyer CJ, and Ingber DE. (1998) Integrin binding and mechanical tension induce movement of mRNA and ribosomes to focal adhesions. *Nature*, 392:730-73

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**Key Words:** *Chromatin, epigenetics, transcription, proliferation, differentiation, leukemia*

Our laboratory is interested in understanding the mechanisms controlling mammalian development and cell differentiation. We study the epigenetic functions of chromatin proteins and transcription factors in control of gene expression in embryonic stem cells, in red blood cells, and in *Drosophila*. Our approaches involve directed gene inactivation and transgenesis in mice and *Drosophila*. We also study control of proliferation and differentiation in red blood cell progenitors and in leukemia cells in which normal development is disrupted. Currently there are two major projects underway in the lab.

**Role of H1 Linker Histones and Chromatin Remodeling Factors in Chromatin Structure, DNA Methylation, the Histone Code, Gene Expression and Development in Mice and *Drosophila*.** Recent studies show that posttranslational modifications of core histones (H2A, H2B, H3, H4) (the Histone Code) play a very important role in control of gene expression. The H1 linker histones are more diverse than the core histones. Mice contain 8 H1 histone subtypes including differentiation-specific and tissue-specific subtypes, whereas *Drosophila* has only one type of H1. H1's are thought to be responsible for the final level of packaging DNA into the compact chromatin structure but we know very little about their role in gene expression and development. We are studying the functional roles of H1 linker histones by inactivating (knocking-out) specific H1 genes in mice and the single H1 in *Drosophila*. We are also reintroducing mutant H1 linker histones into H1 depleted mouse cells and flies, to perform structure-function studies. We have also established a new role for H1 histone in DNA methylation, genomic imprinting and establishment of the histone code. We are also studying the chromatin remodeling factor that assembles H1 histone into chromatin.

**Control of Proliferation and Differentiation in Normal and Leukemic Blood Cells:** In this project we are investigating how cell proliferation and differentiation are coordinated in normal blood cell development and how this coordination is disrupted in leukemia. We have investigating the molecular mechanisms for the cross talk between these two cellular programs in normal and leukemic blood cells. Our studies are focused on the relationships between the master transcription factors that control blood cell development and the cell cycle regulators (cyclins, cyclin-dependent kinases (cdks), cdk inhibitors and RB) that regulate the cell division cycle proliferation. This project includes genome-wide approaches involving chromatin immunoprecipitation and high throughput sequencing (ChIP-Seq) and gene expression profiling by RNA-Seq.

#### **Selected Publications:**

Willcockson, M. A., S. E. Heaton, C. N. Weiss, B. A. Bartholdy, Y. Botbol, L. N. Mishra, D. S. Sidhwani, T. J. Wilson, H. B. Pinto, M. I. Maron, K. A. Skalina, L. N. Toro, J. Zhao, C. H. Lee, H. Hou, N. Yusufova, C. Meydan, A. Osunsade, Y. David, E. Cesarman, A. M. Melnick, S. Sidoli, B. A. Garcia, W. Edelmann, F. Macian and A. I. Skoultchi (2021). "H1 histones control the epigenetic landscape by local chromatin compaction." *Nature* 589(7841): 293-298.

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### **Glycan Functions in Development, Spermatogenesis and Notch Signaling**

Glycosylation is the most abundant and varied post-translational modification of proteins and is a critical factor in regulating their biological functions. The complement of glycans that may be produced by an organism is called the GLYCOME. Changes in glycans expressed on the cell surface occur during development and differentiation. Specific glycans on Notch receptors modulate signal transduction by Notch ligands. This is a novel paradigm of signal transduction whereby the transfer of a single sugar residue alters the ability of Notch receptors to signal. We are using cell-based glycosylation mutants, Notch signaling assays, glycosyltransferase gene knockout mice, and biochemical approaches including MALDI-TOF mass spectrometry, to identify biological functions of growth factor receptor and Notch glycans, and the underlying mechanisms by which glycans mediate biological events.

Notch receptors span the cell membrane. When a Notch ligand like Delta or Jagged on a neighboring cell, binds to a Notch receptor, it induces cleavage of Notch extracellular domain, followed by a second cleavage that releases Notch intracellular domain. The Notch intracellular domain goes to the nucleus and activates target genes that ultimately lead to a change in cell fate or cell growth control. Using a CHO glycosylation mutant that adds few O-fucose glycans to Notch extracellular domain, we showed that Notch signaling is markedly reduced when fucose is limiting. Using a panel of different CHO glycosylation mutants developed in this lab, we showed that inhibition of Notch signaling by the Fringe glycosyltransferase requires the addition of a Gal residue to O-fucose glycans on Notch. We are continuing to use Notch signaling assays to define the mechanisms of action of Fringe and other glycosyltransferases that modulate Notch signaling. We are also targeting glycosyltransferase genes that encode enzymes that modify Notch in the mouse and generating Notch mutants that cannot accept an O-fucose glycan at a specific site in Notch. Mice lacking O-fucose in the ligand binding domain have defective T cell development and are being investigated for other immunological defects. Mice lacking the three Fringe activities are affected in T and B cell development. The most recent modification of Notch is by O-GlcNAc and we are now exploring its functions in the regulation of Notch signaling in mammals.

We are also investigating roles for glycans in reproduction. We have found that MGAT1 and complex N-glycans are essential for spermatogenesis and male fertility, and we are testing the hypothesis that they play an important role in spermatid/Sertoli cell interactions. Small molecule inhibitors are being sought using an in silico docking screen of ~1 million compounds. Candidates will be tested in cell-based assays and ultimately in mice for contraceptive effects. We have shown that deletion of the glycoprotein basigin (CD147) in spermatogonia gives rise to a similar block in spermatogenesis and are investigating whether the N-glycans on basigin are responsible for that phenotype. We are also examining the consequences of deleting MGAT2 in spermatogonia to allow hybrid but not complex N-glycan synthesis.

Finally, Chinese hamster ovary (CHO) cell glycosylation mutants developed in this laboratory are used by us and many academic and biotech laboratories for a variety of purposes including to develop new methods such as a novel approach to tracking glycan epitopes on the cell surface, a method to analyse glycans expressed using scRNA-seq termed sgRNA-seq, assays for Notch and growth factor signaling, and assays to characterize glycosylation mutations that cause rare congenital disorders of glycosylation (CDGs).

### **Recent Publications**

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### **Molecular Regulation and Therapeutic Targeting of Pre-Cancerous and Cancer Stem Cells in Hematopoiesis and Leukemogenesis**

Hematopoiesis maintains a life-long supply of the entire spectrum of highly specialized blood cells dependent on systemic needs. This process relies on a tightly regulated balance of self-renewal, commitment, and differentiation of a small number of pluripotent hematopoietic stem cells (HSC).

Recent experimental evidence has shown that acute myeloid leukemias (AML) and myelodysplastic syndromes (MDS) arise from transformed HSC and committed progenitors. Cell-intrinsic as well as cell-extrinsic aberrations give rise to a highly diverse pool of pre-leukemic stem cells (pre-LSC), preceding the formation of fully transformed leukemia stem cells (LSC). Pre-LSC as well as LSC are characterized by a relative resistance to chemotherapy and thereby contribute to treatment failure. As a consequence, and despite the use of poly-chemotherapy and newer agents that transiently reduce the tumor burden, relapse continues to be the most common cause of death in most subtypes of AML and MDS. Defining the molecular characteristics, heterogeneity, and regulatory mechanisms governing pre-LSC and their subclonal diversity, dynamics, and progression to fully transformed LSC is critical to understanding the genesis of leukemia and to developing therapeutic strategies by which these cells can be eradicated.

Recent findings from our own group and others have demonstrated a critical role of key transcriptional regulators, chromatin-remodeling factors, and mediators of aberrant signaling in the genesis and function of pre-LSC and LSC in AML and MDS in mouse and human model systems.

The goal of our research is to delineate critical mechanisms in HSC that drive formation, progression, and therapeutic resistance of pre-LSC and LSC. To identify and functionally study implicated pathways we are utilizing stem and progenitor cell subsets isolated by means of multi-parameter high-speed fluorescence-activated cell sorting (FACS). We are using experimental tools including at the single-molecule and single-cell level, as well as molecular biological methods for forced expression or inactivation of molecular targets, followed by in vitro as well as in vivo assays for stem and progenitor cell functions including murine transplantation models. This allows for assessing the function of candidate mechanisms in normal and leukemic stem cells. We are studying murine genetic models as well as primary human samples from patients with pre-leukemic conditions and leukemia. Our studies aim at the development of targeted, pre-LSC- and LSC-directed therapies.

#### **Project areas in the lab include:**

- Mechanisms of leukemia pathogenesis at the (pre-leukemic) stem cell level
- Identification and study of novel molecular mechanisms and pathways governing normal and malignant hematopoiesis, including stem cell subclonal heterogeneity, dynamics, and competition (focusing on transcription and signaling, incl. single-molecule and single-cell studies)
- Development and preclinical testing of novel therapeutics targeting aberrant stem cells
- Translational computational biology (e.g. integrated analysis of WGS, scRNA-seq, ChIP-seq, CUT&TAG data etc.; including from longitudinally sampled, sorted / single stem cells from patients and mouse genetic models)



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## **MECHANISMS OF STEM CELL AGING AND TRANSFORMATION**

Key Words: *Hematopoietic stem cells, cell fate determination, hematopoietic malignancies.*

Hematopoietic stem cells (HSC) maintain multi-lineage blood formation throughout our lifetime. Balancing stem cell regeneration and differentiation commitment to produce mature blood cells is quintessential for a healthy hematopoietic system. Dysregulation of such HSC fate determination processes can lead to loss of immune function, bone marrow failure, and malignant transformation during aging. Up to date, very little is known about the molecular events driving age-related HSC changes and how they contribute to disease.

Understanding age-associated molecular alterations will not only uncover fundamental mechanisms guiding function of HSC, but may also allow for therapeutic intervention to “rejuvenate” aged hematopoietic systems and possibly even prevent age-associated hematopoietic diseases. **Our mission is to clarify the central mechanisms establishing and guarding sustained hematopoietic stem cell function, particular those that drive leukemogenesis, if disrupted.** We develop innovative genetic mouse models, use *ex vivo* and *in vivo* primary mouse and human stem cell assay systems, exploit lentiviral gene transfer, and apply state-of-the-art molecular biology and next generation sequencing techniques.

### **Identification of novel molecular safeguards of adult and cancer stem cells**

Teamed-up with Dr. Ana Maria Cuervo (Dept. of Developmental & Molecular Biology), leader in highly precise protein degradation and aging biology, we have recently discovered an important role for chaperone-mediated autophagy in the maintenance of functional HSC (*Dong et al., Nature 2021*). Current efforts investigate the role of this and other stress-related molecular defense mechanisms in leukemic stem cell evolution and maintenance.

### **The labile iron pool as a rheostat for stem cell function**

Our recent work has uncovered a key role of the amount of readily accessible intracellular iron (termed labile iron pool, LIP) in instructing HSC self-renewal (*Kao et al., STM 2018*). We have been investigating the precise molecular mechanism of action, particularly focusing on metabolic and non-enzymatic molecular pathways relying on iron – uncharted territory for healthy as well as leukemic stem cells.

### **Gene expression program erosion in aging stem cells and leukemia**

Our past work has demonstrated a causative role of even minimal dosage alterations of a key transcription factor instructing hematopoiesis, PU.1, observed in hematopoietic stem cell aging to myeloid leukemia evolution (*Will et al., Nat Med 2015*). Our current efforts focus on understanding (1) how such slight deviations from optimal PU.1 dosage lead to the erosion of PU.1-dependent gene expression programs, and (2) how PU.1 gene expression networks functionally cooperate with age-associated inactivation of epigenetic regulators, e.g. TET2 (*Aivalioti et al., Blood Cancer Disc 2022*) and DNMT3A.

### **Improving stem cell-directed therapies**

We are actively engaging with commercial research partners to test and evaluate novel therapeutic options for patients with hematologic malignancies (*Will et al., Blood 2012; Kao et al., STM 2018; Shastri et al., JCI 2018, Okoye-Okafor et al., JEM 2022*).

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Full list of publications can be found at:

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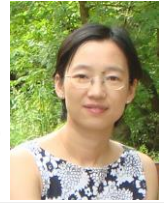
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### **Transcription Regulation and Cell Signaling Control in Normal B/T Cells and Lymphomas**

Molecular pathogenesis of lymphomas situates at the crossroad of lymphocyte development, cancer genetics, transcription regulation, and cell signaling. Thus, we constantly draw upon the most recent advances in these fields to address mechanism questions that are related to lymphoma initiation and development. As each lymphoma entity often corresponds to a specific B/T cell activation/differentiation state that is phenotypically “frozen” by the malignant transformation process, our lymphoma-related studies also provide valuable insights to the regulatory mechanisms that govern the normal immune system. Our research has three major goals: to better understand mature B and T cell development in molecular terms, to decipher how this process is perturbed during lymphomagenesis, and to develop mechanism-based novel therapies to improve patient outcome.

The germinal center (**GC**) response is a very important B cell development stage that has the unique property of generating high affinity antibodies and B cell memory. Because dysregulated GC responses contribute to the development of B cell lymphomas and autoimmune diseases, in-depth understanding of the control mechanisms governing the GC response has both immunological and clinical implications. GCs are dynamic and specialized structures in the secondary lymphoid organs where the B cell genome is subject to two types of genetic alterations catalyzed by AID (activation induced cytidine deaminase), e.g. Ig class switch recombination and somatic hypermutation. Prior to their GC exit, B cells bearing mutated surface Ig molecules undergo positive and negative selections through interaction with two other types of cells in the GC, e.g. follicular dendritic cells follicular T helper (**Tfh**), and T follicular regulatory (**Tfr**) cells. Only the fittest B cells are licensed to terminally differentiate into memory or plasma cells. At the single cell level, the acquisition and termination of GC phenotype is the coordinated transcriptional response to various extracellular and intracellular stimuli; yet the precise sequence and nature of events that orchestrate this process is incompletely understood. We are particularly interested in the roles of two transcriptional factors, BCL6 and STAT3, both are known to play pivotal roles in fate specification and function of GC B cells, Tfh, and Tfr cells.

Our previous studies have revealed novel mechanisms that govern the expression and activity of BCL6, and demonstrated the importance of functional interactions between BCL6 and several cell signaling pathways including RhoA, NF- $\kappa$ B, and Jak/STAT3. In recent years, a central focus of our B cell lymphoma work was on the IL-6/Jak/STAT3 signaling pathway. With the help of our collaborators, we characterized expression regulation of STAT3, cause and consequences of its aberrant activity in diffuse large B-cell (**DLBCL**) development and therapeutic response. Functional contribution of this pathway to normal plasma cell maturation was also investigated.

Our current investigation is focused on the pathogenesis and immunologic features of adult T-cell leukemia/lymphoma (**ATLL**). ATLL is a disease of malignant CD4+ T cells that develops in 4-5% of HTLV-1 carriers after an asymptomatic phase that can persist for decades. The medium survival of ATLL patients diagnosed in North America is only about 7 months, pointing to an urgent

unmet need in the therapeutic space. In a recently study published in *Blood*, we demonstrated that ATLL patients diagnosed in North American (**NA-ATLL**) have a distinct genomic landscape compared to the Japanese cohort (**J-ATLL**). In particular, NA-ATLL is characterized by a much higher frequency of prognostic epigenetic mutations and is targetable preclinically with DNA demethylation drugs. Ongoing studies in our laboratory support the concept that NA- and J-ATLL share similarities in clinical behavior and pathogenesis but also have clear distinctions. Taking advantage of the fact that the Montefiore Medical Center follows one of the largest groups of ATLL patients in the U.S, we have built up a sizable NA-ATLL Biobank, generated and characterized a number of novel NA-ATLL cell lines and PDX models to support our laboratory studies.

#### We are currently pursuing the following research questions:

1. How does BCL6 contribute to the pathogenesis and cell identity of NA-ATLL?
3. What is the genetic and clonal evolution basis that may contribute to the differences between NA- and J-ATLL?
4. How do the ATLL cells suppress the function of normal T cells to achieve immune escape?

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