LUDWIG CENTER | MIT

Ludwig Center at MIT: Updates from Fiscal Year 2023 (July 1, 2022 – June 30, 2023)

The ***Ludwig Center at MIT*** is focused on understanding and addressing the processes associated with malignant progression, in particular the mechanisms that allow cancer cells originating in primary tumors to disseminate and ultimately form metastatic colonies. This multi-step process involves a series of cell-biological and biochemical changes in malignant cells, which are being investigated at several levels. Our research includes identifying and characterizing the biological determinants that allow disseminated cancer cells to gain a foothold in foreign tissue microenvironments, and then to succeed in spawning rapidly growing metastatic colonies.

The work of Ludwig Center members, and their laboratories, is greatly enhanced by the shared sense of mission and community fostered by a program of regular engagement and support from Center colleagues, administrators, and by research and financial resources. The impact of Ludwig Center support can be seen most notably in the research productivity of Center members. Research and programmatic highlights, along with publications and expenditures, are outlined below.

CENTER FACULTY, TRAINEES & COMMUNITY

No changes in membership occurred at the Ludwig Center at MIT during this fiscal year. Drs. Weinberg and Jacks continue to serve as co-directors, while Drs. Francisco J. Sánchez-Rivera and Yadira Soto-Feliciano remain as pilot members. The other active Center members are Drs. Bhatia, Hemann, Spranger, Weissman, Lees, Manalis, and Vander Heiden.

After several decades as a distinguished cancer researcher—honored most recently with the 2022 Lasker Award, recognizing his work on integrins—Ludwig Center at MIT founding member Dr. Richard Hynes will retire at the end of this year. However, he will still have an important presence and footprint in guiding research efforts.

All investigators from the Ludwig Center at MIT have continued to partner with each other and with members of Ludwig Centers at other institutions. Intra-Center collaborations are facilitated in part by community events and meetings, including the annual MIT Ludwig Center Retreat, which was held on May 30, 2023, at MIT's Endicott House. The event included 11 talks from trainees across all active member laboratories, as well as 21 poster presentations, providing numerous interactive opportunities to spark new collaborations.

Inter-Center collaborations this year included several with the Ludwig Center at Harvard, such as ongoing work between Dr. Jacks and Drs. Sandro Santagata and Peter Sorger, and partnerships between the Vander Heiden lab and the Jain and Polyak teams. Dr. Vander Heiden has also initiated a new effort with fellow Ludwig Center at MIT member Stefani Spranger and Ludwig Center at Oxford member Benoit Van den Eynde, via the Ludwig Immunometabolism Initiative.

FACULTY AND TRAINEE AWARDS

Highlights of faculty awards and honors received by Ludwig Center members over the past year include:

Name	Awards and Honors
Richard Hynes	Lasker Award
Tyler Jacks	St. Jude Children's Research Hospital, Vince Kidd Memorial Mentor of the Year Award
Sangeeta Bhatia	Australian Academy of Technology and Engineering, Foreign Fellow

- ***Ludwig Graduate Fellowships*** were awarded to five graduate students:

Name	Project Title
Christina Cabana	"Leveraging *ex vivo* transformed AT2 organoids to investigate the role of the microenvironment in lung adenocarcinoma progression and evolution"
Megan Hoffman	"Investigating SHP2 BioPROTACs as Anti-Cancer therapies"
Caleb Perez	"Leveraging CAR libraries and single-cell omics to deeply characterize CAR signaling space"
Yichen Xiang	"Targeting the Pediatric Cancer Driver PAX3-FOXO1 Fusion Protein"
Daniel Zhu	"Contextualizing malignant pancreatic cell states with spatial modeling of single-cell transcriptomics"

- ***Ludwig Center Postdoctoral Fellowships*** were awarded to eight researchers:

Name	Project Title
Ryuhjin Ahn	"Deciphering cell-type-specific signaling by mass spectrometry-based proteomics"
Elena Cambria	"Deciphering the role of tumor cell mechanical memory in metastasis"
Chuyi Chen	"Creating a stable cross-kingdom colonic interface to study obligated anaerobes in colorectal cancer"
Keith Eidell	"Cytoskeletal-Mediated Resistance to CAR-T cell Treatment"
Saleh Khawaled	"Impacts of aging on intestinal cancer initiation and progression"
Jan-Georg Rosenboom	"An intratumoral hydrogel platform that enables image-guided controlled delivery of immunotherapy drugs"
Tahoura Samad	"Engineering microbially-inspired nano-antidotes to neutralize antibody-drug conjugate toxicity"
Maria Ullo	"Dissecting the interplay between epidermal growth factor receptor (EGFR) spatiotemporal organization and PI3K signal transduction"

For more updates on Ludwig Center activities, please visit https://ludwigcenter.mit.edu/.

RESEARCH HIGHLIGHTS

Selected projects supported by the Ludwig Center at MIT:

Sanchez-Rivera Laboratory: Cancer is a disease characterized by genomic alterations that invariably lead to unabated cellular growth. Perhaps the most prominent of these mutations occur within the *TP53* gene, commonly resulting in loss of function for the tumor suppressor protein, p53. As personalized medicine begins to converge with technological advances in single cell sequencing and functional genomics, it has become quite clear that not all mutations within the same gene are created equal. Indeed, while many tumor models leverage *TP53* deletions, some p53 mutants directly confer proliferative advantages or manifest neo-morphic attributes that operate through distinct mechanisms. Many other clinically observed p53 variants have simply never been studied within a representative context. Therefore, it is imperative that scientists and clinicians evaluate the tumor suppressive or oncogenic role of each p53 variant systematically and precisely.

By integrating high-throughput capabilities with the precision and breadth of engineered mutations afforded by prime editors of DNA, the Sanchez-Rivera laboratory has successfully assembled a generalizable system that models and explores the unique biological perturbations regulated by individual p53 mutants. Briefly, cancer cells are co-infected with vectors encoding the prime editor and its associated prime editor guide RNAs (pegRNA) that contain the complementary templates—optimized via an in-house computational pipeline—for introducing specific p53 mutations. Following selection and sequence validation, subsequent cell growth and migration assays are performed on each engineered variant based on the pegRNA. From these initial proof-of-concept experiments, they identified an uncharacterized variant of TP53 that was found to display enhanced metastatic properties. Preliminary data for other variants with nucleotide changes localized to the oligomerization domain of p53 exhibited phenotypes not consistent with previously described models of exogenous *TP53* expression. These findings not only illuminate understudied consequences of p53 mutants but also highlight the importance of gene dosage and the epigenomic milieu in accurate cancer models.

In addition to pursuing the mechanistic basis for p53 variant discrepancies across lung and pancreatic cancer via precision prime editing, orthogonal DNA engineering technology in the form of base editors is being deployed to study how oncogenic variants in melanoma influence immune evasion. Ongoing partnerships with the Hemann and Soto-Feliciano laboratories similarly incorporate genome editing techniques to understand the role of specific cancer alleles, including those encoding mutated histones, in the context of therapeutic resistance in blood cancers. In collaboration with Tyler Jacks, the Sanchez-Rivera group was also able to adapt their DNA editing platform for *ex vivo* pancreatic organoid engineering. Overall, the methodology of high-throughput precision genome editing developed by the Sanchez-Rivera laboratory will yield substantial insights into how functional cancer genomics can deconstruct tumor heterogeneity for optimal patient-therapy stratification.

Relevant publication(s):

1. Ely, Z. A.*, Mathey-Andrews, N.*, Naranjo, S., Gould, S. I.†, Cabana, C. M., Mercer, K. L., Rideout III W. M., Newby, G. A., Cervantes Jaramillo, G., Holland, K., Randolph, P. B., Freed-Pastor, W. A., Davis, J. R., Westcott, P. M. K., Anzalone, A. V., Pattada, N. B., Sánchez-Rivera, F.J., Liu, D. R., Jacks, T. (2023). **A prime editor mouse for modeling**

a broad spectrum of somatic mutations *in vivo*. *Nature Biotechnology*. DOI: https://doi.org/10.1038/s41587-023-01783-y. *Equal contribution.

2. Gould, S.I.†, Sánchez-Rivera, F.J.# (2022). **PEGG: A computational pipeline for rapid design of prime editing guide RNAs and sensor libraries**. bioRxiv. DOI: https://www.biorxiv.org/content/ 10.1101/2022.10.26.513842v3.

Spranger Laboratory: Most immune checkpoint blockade (ICB) therapies work by inhibiting immunosuppressive proteins expressed on the surface of immune cells, notably CTLA-4 or PD-1. By disrupting the function of these signaling molecules, cytotoxic T cells can more effectively kill cancer cells. While ICB therapy has performed quite well in certain hematological malignancies, the majority of patients with solid tumors do not receive a durable response from ICB treatment. Consequently, much research attention is focused on where this process goes wrong and how to restore ICB sensitivity.

The Spranger laboratory studies anti-tumor immunity and ICB response from the perspective of the tissue crosstalk between the tumor and adjacent tumor-draining lymph nodes (tdLN), as lymph nodes are the major site of priming and converting naïve T cells into functional cytotoxic T cells that can kill cancer cells. Initial evidence from the Spranger group revealed that orthotopic lung tumors displayed poor ICB responses compared to subcutaneous lung cancer models, which related to inadequate T cell priming specifically within lung proximal tdLNs. Importantly, this T cell population did not express typical exhaustion markers and thus represented a new T cell state. Over the past year, the Spranger laboratory sought to further explain why tdLNs adjacent to orthotopic lung tumors comprise an environment that uniquely produces ineffective T cells. To address this question, they turned their sights on dendritic cells—critical players in priming T cells against tumor antigens. Utilizing flow cytometry gated against markers of dendritic cell function, they found that dendritic cells specifically derived from the lung proximal tdLNs expressed lower levels of immunostimulatory molecules such as CD80, CD86, and IL-12 when compared to dendritic cells derived from the subcutaneous tdLN. As these protein markers are known to be downregulated in dendritic cells upon interaction with regulatory T cells (Tregs), the Spranger laboratory then examined the extent to which Tregs could be involved in this immunosuppression by repeating the experiments in a mouse model depleted of Tregs. In these depleted Treg backgrounds, dendritic cell activation markers were restored, and antitumor T cell priming was rescued following ICB therapy. These results provide a mechanistic understanding for ICB insensitivity in lung cancer that is dependent on Treg activity and may have significant clinical implications for enhancing the response to ICB therapy in solid cancers more generally.

Future work from the Spranger laboratory will look to uncover the molecular processes governing activated Tregs in lung proximal tdLNs. In collaboration with the Vander Heiden group, the Spranger laboratory will also evaluate nutrient availability within distinct tdLNs and its potential role in regulating Treg-mediated suppression of dendritic cells following ICB therapy.

Relevant publication(s):

1. Zagorulya M, Yim L, Morgan DM, Edwards A, Torres-Mejia E, Momin N, McCreery CV, Zamora IL, Horton BL, Fox JG, Wittrup KD, Love JC, Spranger S. **Tissue-specific**

abundance of interferon-gamma drives regulatory T cells to restrain DC1-mediated priming of cytotoxic T cells against lung cancer. *Immunity*. 2023 Feb 14;56(2):386-405.e10. doi: 10.1016/j.immuni.2023.01.010. Epub 2023 Feb 2. PMID: 36736322

Weissman Laboratory: Fundamentally, cancer is a progressive malignancy that continually proliferates and adapts to its surroundings. While much effort has gone into classifying tumors by their driver mutations or expression of key genes at the time of diagnosis or treatment follow-up, disproportionately less work has been performed on broadly categorizing and modeling the adaptative tumor cell states that evolve across time and space (i.e., tissue localization). As mutational data does not capture the dynamic state of gene expression nor consistently provide a clear explanation for therapy failure, it is critically important that clinical assessments start to incorporate information on the evolutionary nature of a cancer, including its propensity to generate resistant or highly metastatic subpopulations. Therefore, technologies that can reliably track tumor cell states will be invaluable for more precise treatment strategies and outcome predictions.

To develop the necessary tools to study tumor evolution, the Weissman laboratory utilizes CRISPR/Cas9-based genome engineering to insert unique DNA sequences, or “barcodes,” into the genomes of cells. Once a population of cells is generated where each cell possesses a distinctive genetic identifier, scientists can essentially record and trace the lineage of a particular cell clone as it divides with matching gene expression analysis. Over the past year, the Weissman laboratory, in collaboration with the Jacks laboratory and the Sankaran group at Harvard Medical School, refined their tracing approach by leveraging natural barcodes within mitochondrial DNA, which expanded the range of cells that could be mapped to a particular lineage and be utilized for more robust single-cell analyses. This updated method was deployed in hematopoietic stem cells (HSC) to model distinct physiological states and functional heterogeneity over time, delineated across gene expression and chromatin accessibility. In addition to the general utility of the deepened barcoding platform for combinatorial lineage tracing and cell state analyses, this temporal information on HSC clonality will serve as a critical reference point for modeling blood cancer cell evolution.

For the upcoming year, the Weissman laboratory is expanding the practical number of barcodes that can be generated via prime editing. In addition, by utilizing fluorescent in situ hybridization techniques that allow for imaging capabilities, the Weissman laboratory is planning to incorporate spatial information into their lineage tracing system. As the tumor microenvironment is a complex arena of multiple cell types and cell-cell interactions, the overall goal will be to elucidate how tumor cell evolution is directed in 4-D, providing a more comprehensive picture—beyond genomics—to inform cancer treatment strategies.

COMPLETE LIST OF PUBLICATIONS (7/2022 – 6/2023)

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3. Diehl FF, Sapp KM, **Vander Heiden MG**. The bidirectional relationship between metabolism and cell cycle control. Trends Cell Biol. 2023 Jun 27:S0962-8924(23)00110-1.

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11. Zagorulya M, **Spranger S**. Once upon a prime: DCs shape cancer immunity. Trends Cancer. 2023 Feb;9(2):172-184. doi: 10.1016/j.trecan.2022.10.006. Epub 2022 Nov 7. PMID: 36357313

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EXPENDITURES

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