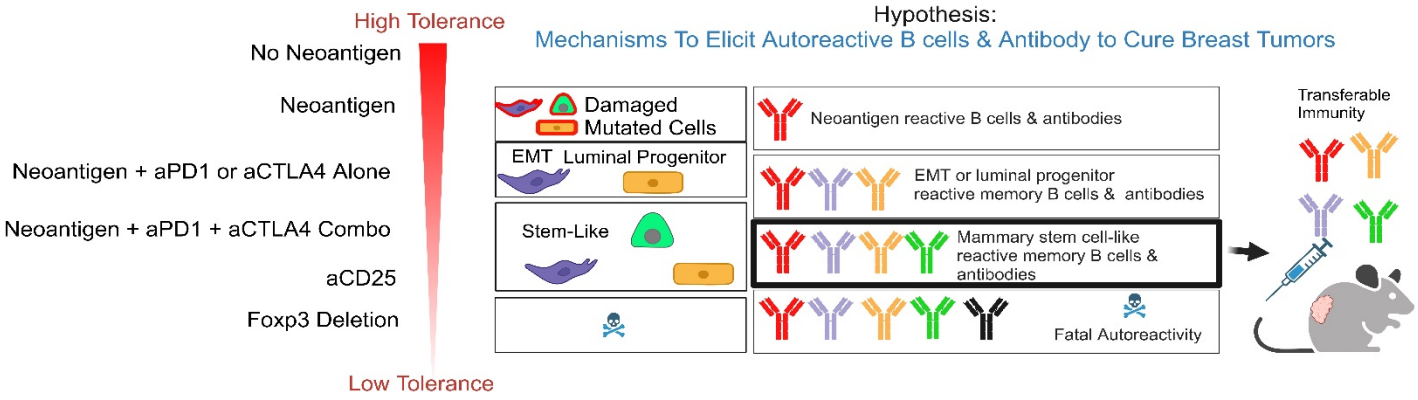
Unleashing and Transferring Autoimmunity to Cure Metastatic Triple Negative Breast Cancers. Pew Stewart Cancer Scholars Letter of Intent

Daniel Hollern PhD, Assistant Professor



Immunotherapies targeting T cell immune checkpoints provide clinical benefit but too often fail to completely eliminate tumor cells; likely related to requirements for neoantigen expression by tumor cells (ie- tumor cell expression of mutated DNA) which are often unstable. Becuase of the tight links between neoantigen requirements and patient outcome, research has intensely focused on the study of adaptive immune responses. Yet, hidden among adaptive immune responses to cancer, in our study we discover autoreactive pathways that cure mice of aggressive metastatic chemotherapy resistant cancers.

Adaptive immunity functions by germinal center B and T cell responses in lymph node follicles and in tertiary lymphoid structures. In germinal centers, B cells somatically hypermutate their B cell receptors, optimizing cellular and humoral immunity to foreign or mutated self-antigens (neoantigens). Non-adaptive responses to antigen use an extrafollicular pathway in lymhpoid tissues that pre-encodes receptors and antibody responses in B cells. Yet, extrafollicular B cell responses show tight links to autoimmunity, thus, concerns on the severity of immune related adverse events have made this pathway overlooked anti-tumor treatments.

Leveraging autoreactive extrafollicular B cell and humoral responses would not be problematic if we could safely turn on autoimmunity for a certain period of time and use it to drive cytotoxic immunological rejection of tumor cells. Since breast cancer cells embody also normal mammary wound healing cells, autoreactivity may be limited to mammary epithelial cells and risk of systemic autoimmunity may be minimal in this context. By exploting autoreactivity we could target a much broader population of tumor cells, without being limited by their expression of neoantigens. Yet, knowledge gaps in the regulation and function of autoreactive B cell and humoral immunity limit development of safe strategies and translation of these breakthrough concepts to patients.

Adding support to this exciting premise, our data show that a pre-encoded B cell population can be unlocked by checkpoint modulation to elicit systemic humoral and immune responses that eliminates chemotherapy resistant neoantigen negative tumor cells. The pre-encoding of this B cell population and humoral response is extremely notable as it infers reliable accessiblity and generalization. With our groundbraking finding we envision the possibility for a novel type of immunotherapy that could overcome previous limitations of tumor cell immune escape. Yet, how to safely exploit autoreactive B cells and humoral responses that have the potential to reject a diversity of tumor cells is still not an established regimen.

These data lead us to hypothesize that B cell and humoral autoreactivity can be tuned by using checkpoints T cells use to alter B cell antigen tolerance. Aim 1 will test this hypothesis and reveal modes of CD4 T cell alteration and function leading to curative B cell and humoral autoreactivity to cancers. Since the B cell responses we have observed in our pilot experiment resembled memory cells, we hypothesize that they can provide cell based therapies for tumor rejection. This motivates Aim 2 to uncover how autoreactive B cell responses, particularly memory B cells function in tumor cell rejection. Likewise, we find that autoreactive antibody transfer can drive rejection of tumor cells. This leads us to hypothesis that antibody has direct tumor cell killing capacity but also broader impact by reprogramming tolerance and sustaining autoreactive rejection of cancer cells. In Aim 3 we will uncover how transfer of systemic antibodies elicit tumor rejection. When this work is complete, we will establish novel rationale for turning autoimmunity against cancer.

Aim 1: Discover mechanisms that regulate B cell and humoral tolerance or rejection of tumor cells.
Aim 2: Determine the extent of functional tumor cell rejection information transferrable by memory B cells.
Aim 3: Determine the extent of functional tumor cell rejection information encoded in humoral immunity.

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CONTACT INFORMATION

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RESEARCH SUMMARY

My research uses a multi-disciplinary approach to investigate mechanisms of response to therapy, immune cell dynamics in human cancers, and mechanisms controlling tumor metastasis. By integrating expertise in functional genomics with mouse models and experimental biology, the goal of my research is to improve treatment strategies for cancer patients.

EDUCATION and TRAINING

Assistant Professor, Salk Cancer Center, NOMIS Center for Immunobiology and Microbial Pathogenesis
The Salk Institute for Biological Studies, La Jolla, California
2021-Current Position

Postdoctoral Research Fellow, Cancer genetics and genomics, 2015-2020
University of North Carolina, Chapel Hill, NC
Mentor: Charles M Perou, Ph.D.
Areas of Study: Computational Genomics, Immunogenomics, Tumor Immunology, Breast Cancer, Testicular Cancer

Ph.D., Cell and Molecular Biology, 2015
Michigan State University, East Lansing
Mentor: Eran R. Andrechek, Ph.D.
Thesis: *A study of breast cancer heterogeneity and molecular mechanisms of metastasis*

Bachelors of Science, Biology, 2009
Grand Valley State University, Allendale, MI

32 PUBLICATIONS

1. Ramos M, Lui A, and **Hollern D**. The Evolving Landscape of B Cells in Cancer Metastasis: Recent Findings and Future Directions. 2023. **Cancer Research**. 83.23 (2023): 3835-3845
2. Swiatnicki, M. R., Rennhack, J. P., Ortiz, M. M., **Hollern, D. P.**, Perry, A. V., Kubiak, R., ... & Andrechek, E. R. (2022). Elevated phosphorylation of EGFR in NSCLC due to mutations in PTPRH. *PLoS Genetics*, *18*(9), e1010362.
3. Mara Gilardi, Monika Ramos, **Daniel Hollern.** B cells secrete GABA, which provokes a pro-tumor immune microenvironment. 2022. ***Cancer Cell***. https://doi.org/10.1016/j.ccell.2021.12.007
4. Celine Laumont , Allyson Banville, Mara Gilardi**, Daniel Hollern**, and Brad Nelson. B cells in cancer: clinical impact, immunological mechanisms and therapeutic opportunities. 2022. ***Nature Reviews Cancer.*** https://doi.org/10.1038/s41568-022-00466-1
5. Mara Gilardi, Robert Saddawi-Konefka, Victoria H Wu, Miguel Angel Lopez-Ramirez, Zhiyong Wang, Fernando Soto, Dana J Steffen, Marco Proietto, Zbigniew Mikulski, Haruka Miki, Andrew B Sharabi, Daniel Kupor, Ricardo Rueda, **Daniel Hollern**, Joseph Wang, and J. Silvio Gutkind. Microneedle-mediated intratumoral delivery of anti-CTLA-4 promotes cDC1-dependent eradication of oral squamous cell carcinoma with limited irAEs. 2022. ***Molecular Cancer Therapeutics***. https://doi.org/10.1158/1535-7163.MCT-21-0234
6. He, Y., Wang, L., Wei, T., Xiao, Y.T., Sheng, H., Su, H., **Hollern, D.P**., Zhang, X., Ma, J., Wen, S. and Xie, H., 2021. FOXA1 overexpression suppresses interferon signaling and immune response in cancer. ***The Journal of Clinical Investigation***
7. Garay, J.P., Smith, R., Devlin, K., **Hollern, D.P**., Liby, T., Liu, M., Boddapati, S., Watson, S.S., Esch, A., Zheng, T. and Thompson, W., 2021. Sensitivity to targeted therapy differs between HER2-amplified breast

cancer cells harboring kinase and helical domain mutations in PIK3CA. ***Breast Cancer Research***, *23*(1), pp.1-17.

8. Bai, F., Wang, C., Liu, X. *et al*. Loss of function of BRCA1 promotes EMT in mammary tumors through activation of TGFβR2 signaling pathway. *Cell Death Dis* **13**, 195 (2022). https://doi.org/10.1038/s41419-022-04646-7
9. Bai, F., Liu, S., Liu, X., **Hollern, D.P**., Scott, A., Wang, C., Zhang, L., Fan, C., Fu, L., Perou, C.M. and Zhu, W.G., 2021. PDGFRβ is an essential therapeutic target for BRCA1-deficient mammary tumors. ***Breast Cancer Research***, *23*(1), pp.1-17.
10. Swarnima Singh, Nigel Lee, Igor Bado, Clark Hamor, Licheng Zhang, Sergio Aguirre, Jingyuan Hu, Yichao Shen, Yitian Xu, Yang Gao, Diego Pedroza, Na Zhao, Shu-Hsia Chen, Ying-Wooi Wan, Zhandong Liu, Jeffrey Chang, **Daniel Hollern**, Charles Perou, Xiang Zhang, and Jeffrey Rosen. Chemotherapy coupled to macrophage inhibition leads to B cell-mediated T cell memory activation and durable triple negative breast cancer regression. ***bioRxiv.*** https://www.biorxiv.org/content/10.1101/2021.02.22.432300v1.abstract
11. Swiatnicki MR, Rennhack JP, **Hollern D**, Perry AV, Kubiak R, Riveria SM, O'Reilly S, Andrechek ER. Elevated phosphorylation of EGFR in NSCLC due to mutations in PTPRH. ***bioRxiv***. 2021 Jan 1.
12. Briana To, Carson Broeker, Jing-Ru Jhan, Rachel Rempel, Jonathan P Rennhack, **Daniel Hollern**, Lauren Jackson, David Judah, Matthew Swiatnicki, Evan Bylett, Rachel Kubiak, Jordan Honeysett, Shams Reaz, Joseph R Nevins, Eran Robert Andrechek. 2021. Insight into mammary gland development and tumor prevention in a newly developed metastatic mouse model of breast cancer. ***bioRxiv***.https://www.biorxiv.org/content/10.1101/2021.09.24.461727v1.abstract
13. Crosby EJ, Acharya CR, Haddad A, Rabiola CA, Lei G, Wei J, Yang X, Wang T, Liu C, Wagner KU, Muller WJ, Chodosh LA, Broadwater G, Hyslop T, Shepherd JH, **Hollern D**, He X, Perou CM, Chai S, Ashby BK, Vincent BG, Snyder JC, Force J, Morse MA, Lyerly HK, Hartman ZC. 2020. Stimulation of Oncogene-Specific Tumor-Infiltrating T Cells through Combined Vaccine and αPD-1 Enable Sustained Antitumor Responses against Established HER2 Breast Cancer. ***Clinical Cancer Research***. https://doi.org/10.1158/1078-0432.CCR-20-0389
14. Garcia-Recio S, Thennavan A, East MP, Parker JS, Cejalvo JM, Garay JP, **Hollern DP**,He X ,Mott KR, Galván P, FanC, Selitsky SR, Coffey AR, Marron D, Brasó-Maristany F, Burgués O, Albanell J, Rojo F, Lluch A, Martinez de Dueñas E, Rosen JM, Johnson GL, Carey LA, Prat A, Perou CM. 2020. ***The Journal of Clinical Investigation***. https://doi.org/0.1172/JCI130323
15. Williams M, Liu X, Zhang Y, Reske J, Bahal D, Gohl T, **Hollern D,** Ensink E, Kiupel M, Luo R, Das R, and Xiao H. 2020. NCOA5 deficiency promotes a unique liver protumorigenic microenvironment through p21WAF1/CIP1 overexpression, which is reversed by metformin. ***Oncogene***. https://doi.org/10.1038/s41388-020-1256-x
16. Selitsky, S.R., Marron, D., **Hollern, D**., Mose, L.E., Hoadley, K.A., Jones, C., Parker, J.S., Dittmer, D.P. and Perou, C.M., 2020. Virus expression detection reveals RNA-sequencing contamination in TCGA. ***BMC genomics***, *21*(1), p.79.
17. **Hollern, D. P**. *et al.* Perou, C.M. 2019. B Cells and T Follicular Helper Cells Mediate Response to Checkpoint Inhibitors in High Mutation Burden Mouse Models of Breast Cancer. ***Cell*** 179,1191-1206.e1121, doi:10.1016/j.cell.2019.10.028
*** Immune Regulation News Top Story * Joseph S. Pagano Award**
18. **Hollern DP,** et al. Perou, C.M., 2019. A mouse model featuring tissue-specific deletion of p53 and Brca1 gives rise to mammary tumors with genomic and transcriptomic similarities to human basal-like breast cancer. ***Breast cancer research & treatment***, *174*(1), pp.143- 155.
19. **Hollern DP,** et al Andrechek, E.R., 2019. E2F1 Drives Breast Cancer Metastasis by Regulating the Target Gene FGF13 and Altering Cell Migration. ***Scientific Reports*** https://doi.org/9:10718.
20. An, Y., Adams, J.R., **Hollern DP (*Co-first author)**, Zhao, A., Chang, S.G., Gams, M.S., Chung, P.E., He, X., Jangra, R., Shah, J.S. and Yang, J., 2018. Cdh1 and Pik3ca Mutations Cooperate to Induce Immune-Related Invasive Lobular Carcinoma of the Breast. ***Cell reports***, *25*(3), pp.702-714.
21. Shen H*, Shi J*, **Hollern DP* (*Co-first author)** , TCGA working group. 2018. Integrated molecular characterization of testicular germ cell tumors. ***Cell reports*** 23.11 (2018): 3392-3406.

22. **Hollern DP,** Swiatnicki MR, Andrechek ER. 2018. Histological subtypes of mouse mammary tumors reveal conserved relationships to human cancers. ***Plos Genetics*** 14: 1 https://doi.org/10.1371/journal.pgen.1007135

23. Mukherjee, A., **Hollern DP**, Williams, O., Rayburn, T., Byrd, W., Yates, C., & Jones, J. D. (2018). A Review of FOXI3 Regulation of Development and Possible Roles in Cancer Progression and Metastasis. ***Frontiers in cell and developmental biology***, 6, 69.

24. Tanioka, M., Mott, K.R., **Hollern, DP**, Fan, C., Darr, D.B. and Perou, C.M., 2018. Identification of Jun loss promotes resistance to histone deacetylase inhibitor entinostat through Myc signaling in luminal breast cancer. ***Genome medicine***, *10*(1), p.86.

25. Acosta, D., Bagchi, S., Broin, P.Ó., **Hollern, DP**, Racedo, S.E., Morrow, B.,Sellers, R.S., Greally, J.M., Golden, A., Andrechek, E. and Wood, T., 2016. LPA receptor activity is basal specific and coincident with early pregnancy and involution during mammary gland postnatal development. ***Scientific reports***, *6*, p.35810.

26. Lunt SY, Muralidhar V, Hosios AM, Israelsen WJ, Gui DY, Newhouse L, Ogrodzinski M, Hecht V, Xu K, Acevedo PN, **Hollern DP**, Bellinger G, Dayton TL, Christen S, Elia I, Dinh AT, Stephanopoulos G, Manalis SR, Yaffe MB, Andrechek ER, Fendt SM, Vander Heiden MG. Pyruvate kinase isoform expression alters nucleotide synthesisto impact cell proliferation. ***Molecular Cell***. Volume 57, Issue 1, 8 January 2015, Pages 95–107

27. **Hollern DP,** Andrechek ER. 2014. A Genomic Analysis of Mouse Models of Breast Cancer Reveals Important Molecular Features of Individual Mouse Models and Relationships to Human Breast Cancer. ***Breast Cancer Research*** 16:R59
*** Editors pick ** Highly accessed *** Top 5 for most downloaded papers in 2014**

28. **Hollern DP,** Honeysett J,Cardiff RD, Andrechek ER. 2014. The E2F transcription factors regulate tumor development and metastasis in a mouse model ofmetastatic breast cancer. ***Molecular and Cellular Biology***.

29. Chen F, Li A, Gao S, **Hollern DP,** VanSickle EA, Williams M, Andrechek EA, Zhang C, Yang C, Luo R, and Xiao H. 2014. Tip30 controls differentiation of murine mammary luminal progenitor to estrogen receptor-positive luminal cell through regulating FoxA1 expression. ***Cell Death and Disease*** 5:e1242

30. Zhou X, **Hollern DP**, Liao J, Andrechek E, and Wang H. 2013. NMDA receptor- mediated excitotoxicity depends on the co-activation of synaptic andextrasynaptic receptors. *Cell Death and Disease* 4, e560

31. **Hollern DP**, Yuwanita I, Andrechek ER. 2012. A mouse model with T58A mutations in Myc reduces the dependence on KRas mutations and has similarities to claudin-low human breast cancer. *Oncogene*. 32(10):1296-304

32. Fujiwara K, Yuwanita I, **Hollern DP**, Andrechek ER. 2011. Prediction and Genetic Demonstration of a Role for Activator E2Fs in Myc-Induced Tumors. *CancerResearch* 71(5): 1924-1932.

GRANTS AWARDED

2024	Identifying pathogen exposures that ignite breast cancer risk and lethality. California Breast Cancer Research Program. Many Pathways to Cancer Award
2023	Identifying the antigens involved in tumor intolerance and autoreactive B cell resposnes. Salk P30
2023	Nicotine exposure in driving breast cancer metastasis. Curebound Discovery Award
2022	Discovering modes of B cell activation to enhance the treatment of metastasis. Susan G Komen Career Catalyst Award
2021	Using B cells to eliminate TNBC liver metastases. Metavivor Early Career Investigator Award
2021	Crosstalk between EMT & immune cells in breast cancer immunotherapy resistance. Pedal the Cause.
2021	Identifying the Role of B cells and Inflammation in Hepatocellular Carcinoma Initiation. Salk P30

2020	Uncovering pathways that control response and resistance of TNBCs to immunotherapy. Salk P30
2015	Ruth L. Kirschstein National Research Service Award (NRSA) Post-doctoral fellowship F32 National Cancer Institute CA210427
2014	Ruth L. Kirschstein National Research Service Award (NRSA) Post-doctoral fellowship F31 National Cancer Institute CA183272
2014	Aitch Foundation Fellow
2013	College of Natural Science Dissertation Continuation Fellowship
2010	Michigan State University, The Graduate School Summer Fellowship

SELECT PUBLIC PRESENTATIONS OF RESEARCH

1. Investigating B cell - T cell interactions to identify therapeutic opportunities for cancer patients. World Vaccine and Immunotherapy Congress. 2021.
2. B Cells and T Follicular Helper Cells Mediate Response to Checkpoint Inhibitors in High Mutation Burden Mouse Models of Breast Cancer. Virtual oral presentation. Deeley Research Centre Seminar Series. 2020.
3. B Cells and T Follicular Helper Cells Mediate Response to Checkpoint Inhibitors in High Mutation Burden Mouse Models of Breast Cancer. Virtual oral presentation. B cells in Cancer UNC-Kings College of London Virtual Symposium. 2020.
4. Apobec3 induced mutagenesis sensitizes murine models of triple negative breast cancer to immunotherapy by activating B-cells and CD4+ T-cells. **Oral Presentation, General assembly, San Antonio Breast Cancer Symposium**, December 2018
5. Gene Expression features of Seminomas, Mixed Tumors, and Kit Mutant Testicular Germ Cell Tumors, Oral Presentation, TCGA Testicular Germ Cell Data Analysis Workshop, November 2015
6. Identifying Genes That Control Breast Cancer Metastasis, Oral Presentation, Aitch Foundation Hidden Key Fashion Show Fundraiser, June 2014
7. Human breast cancer and mouse models; similarities and constraints identified through gene expression patterns. Oral Presentation Michigan State University, Biomolecular Sciences Annual Retreat, August 2013
8. Human breast cancer and mouse models; similarities and constraints identified through gene expression patterns. Oral Presentation, Mammary Gland Biology, Gordon Research Conferences, June 2013

HONORS and AWARDS

2024	Invited session co-chair. Society for the Immunotherapy of Cancer Annual Meeting.
2024	Invited Speaker. Society for the Immunotherapy of Cancer Annual Meeting.
2023	Invited Participant. SITC TLS Consensus Meeting
2023	Invited Speaker. La Jolla Immunology Conference
2023	Organizer and Invited Speaker. Keystone Symposia on Cell & Molecular Biology
2022	Organizer. SITC Deep Dive Webinar.
2021	Invited Speaker. U Mass.
2021	Invited Speaker. World Vaccine and Immunotherapy Congress.
2021	Invited Speaker. Salk Cancer Center Summit
2020	Joseph S Pagano Award. University of North Carolina
2020	Invited Speaker. Deeley Research Centre Seminar Series
2020	Invited Speaker. B cells in Cancer UNC-Kings College of London Virtual Symposium
2018	Invited Speaker. San Antonio Breast Cancer Symposium.
2014	The Aitch Foundation Award
2013	Invited Speaker. Mammary Gland Biology, Gordon Research Conferences
2013	Gordon Research Conference Travel Fellowship
2013	The Graduate School Travel Fellowship
2013	Cell and Molecular Biology Travel Fellowship
2007-2009	Dean's List, Grand Valley State University

2003-2004 Dean's List, Grand Rapids Community College

Research Experience

University of North Carolina, Chapel Hill, *Post-doctoral Research Associate,* 2015 to Present
Breast cancer genetics and genomics
Mentor: Dr. Charles M. Perou, Ph.D., May Goldman Shaw Distinguished Professor of Molecular Oncology

- **Identification of predictive gene signatures and mechanisms of response to immune checkpoint inhibitors**
  - Lead study identifying predictors and mechanisms of response to immune checkpoint inhibitors using preclinical models of triple negative breast cancer.
  - Developed new mouse mammary tumor models to study the immune cell dynamics in triple negative breast cancer.
  - Discovered the necessity of B cells, antibody secretion, and T follicular helper cell function to sustained anti-tumor responses to immune checkpoint inhibitors.

- **The Cancer Genome Atlas (TCGA) project – Testicular Germ Cell Tumors analysis working group**
  - Co-lead author on Integrated Molecular Characterization of Testicular Germ Cell Tumors.
  - Analyzed the immune landscape of testicular germ cell tumors.
  - Identified the BCR and TCR repertoire of testicular germ cell tumors.
  - Identified histological specific mRNA expression (mRNAseq) patterns.
  - Identified elevated kit signaling pathway components in testicular germ cell tumors.

- **Credentialing mouse models for the study breast cancer and the immune microenvironment**
  - Led genomic analyses to characterize mouse models of invasive lobular carcinoma.
  - Led genomic analyses to establish mouse models of basal-like breast cancer
  - Established relationships to human breast cancer
  - Led genomic studies to identify key functional pathways and immune cell subsets

Michigan State University, *Graduate Research Assistant,* 2009 - 2015
Cancer genetics and genomics
Advisor: Dr. Eran Andrechek, Ph.D., Associate Professor with Tenure

- **Development of gene expression signatures of mouse mammary tumor histology**
  - Developed and experimentally validated a series of gene expression signatures capable of predicting mouse mammary tumor histology.
  - Identified relationship of mouse model tumor histologies to activation of key oncogenic pathways.
  - Identified relationship of mouse model tumor histologies to human cancer histologies and molecular subtypes on the basis of transcriptomes and oncogenic pathway activity.

- **Dissecting tumor heterogeneity of breast cancer using mouse models**
  - Developed an expansive database of 26 major mouse models of breast cancer.
  - Determined optimal procedures for adjusting and monitoring batch effects.
  - Identified tumor heterogeneity within models on the basis of gene expression profiles and pathway activity.
  - Identified mouse models that parallel human breast cancer.
- **Discovery of novel mechanisms of metastasis using computational analysis of mouse model and breast cancer transcriptomes**
  - Predicted and validated a role for the E2F transcription factors in breast cancer metastasis using a computational analysis of mouse and human breast tumors and genetic tests.
  - Discovered that loss of E2F1 leads to a reduction in circulating tumor cells
  - Identified that loss of E2F1 reduces tumor angiogenesis concomitant with reduced expression of multiple E2F target genes involved genes involved in the hypoxia response.
  - Used CRISPR gene editing to knockout FGF13 and validate a role for FGF13 in metastasis.

Grand Valley State University, *Undergraduate Research Assistant,* 1998-2000
Plant Cell & Developmental Biology
Advisor: Dr. Sheila Blackman, Ph.D., Professor
- Performed immunofluorescence staining to assess cytoskeleton structure of cell cultures of

Daucus carota in response to integrin-binding peptide, RGD.

SERVICE and LEADERSHIP

B Cells in Cancer Consortium Co-Founder

2021 DOD Study Section Reviewer CON-GC-BC

2021 DOD Study Section Reviewer PB-6

Ad hoc reviewer: Scientific Reports, Nature, Nature Medicine, Cell,

Journal of Immunology , Clinical Cancer Research
The Cancer Genome Atlas (TCGA, Testicular Germ Cell Tumors 2015-2018)
AACR

MANAGEMENT EXPERIENCE and TRAINING

- Principle investigator
- Mentored 1 MD / post doc to independence
- Mentored 1 post doc to industry
- Mentor 2 Grad Students
- Mentor 2 Post docs
- Supervise 1 Research Assistant
- 1 part time administrator
- Completed Mentor Training Group for Junior Faculty
- Completed PiBS T32 Mentorship Training

Overview: The ability of cancer cells to rewire cellular processes is a powerful way to enhance disease progression. A hallmark of many cancers is the rewiring of signal transduction pathways to sustain cancerous phenotypes. The PI3K/Akt and Wnt-signaling pathways are some of the most commonly altered pathways in cancer linked to drug resistance and increased proliferation, differentiation, and metastasis[1]. Recently, the activation of these pathways by non-receptor signal transducers has emerged as a powerful control mechanism. Girdin/GIV and Daple are non-receptor signal transducers and guanine exchange factors (GEFs) that regulate these pathways[2,3]. Their GEF activity and binding to Gα subunits of heterotrimeric G proteins are affected by Akt-driven phosphorylation, cellular localization, and other interactions[4]. However, little is known about the molecular architecture of these proteins, prompting structural studies to fully understand their function.

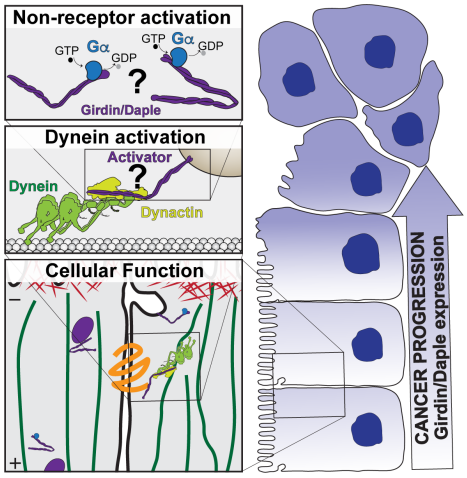

Figure 1. Non-receptor and dynein-driven rewiring of cancer progression.

Girdin and Daple colocalize with cytoplasmic dynein-1 (dynein). Dynein is an essential microtubule-based motor with mitotic and cargo-transporting functions. The dynein complex (dynein/dynactin) requires binding to an "activator" that initiates its motility and links it to cargoes, including endocytic vesicles. I have shown that the Hook family of dynein activators links dynein to other motors and multiple cargos, emphasizing the importance of activators in regulating dynein function[5,6]. Girdin and Daple share homology with the Hook proteins and are proposed to interact with dynein, yet the molecular details of these interactions remain unknown. *I hypothesize that these proteins activate dynein, which is critical for localizing Girdin and Daple to the sites of G-protein activation and subsequent regulation of signaling pathways (**Figure 1**).*

Goals: The goal of my research is to understand how intracellular transport can be rewired to sustain cancerous phenotypes. Using CryoEM, including time-resolved approaches and cross-linking mass spectrometry, **we will determine the molecular architecture of Girdin and Daple.** This will allow us to unravel the three-dimensional structures of Girdin and Daple and the molecular mechanism of their interaction with G-α during nucleotide exchange, potentially capturing intermediate states of GEF activity critical for future rational drug design to treat cancer. **We will decipher the mechanism of Girdin and Daple interaction with dynein.** Using in vitro motility assays with crude cell extracts from cancer cell lines or purified proteins, we will determine if Girdin and/or Daple bind and activate the dynein complex. We will use live-cell imaging in cancer cell lines of dynein and its cargoes in the presence of different Girdin or Daple constructs to determine the cellular role of these interactions and their contribution to cancerous phenotypes (i.e., growth and proliferation).

Significance: The significance of our work lies in deciphering the mechanisms by which non-receptor signal transducers regulate signaling pathways and link to intracellular transport. Our discoveries will not only provide the needed information to develop future therapeutics but will also explain the molecular mechanisms of these proteins' functions that apply to other cell types and disease models. Given my previous experience in the fields of cancer and intracellular transport and cutting-edge experimental toolset, I am ideally positioned to successfully execute this project.

References:

1. Y. Yeoh, T. Y. Low, N. Abu, P. Y. Lee, Regulation of signal transduction pathways in colorectal cancer: implications for therapeutic resistance. *PeerJ* **9**, e12338 (2021).
2. N. Aznar, K. K. Midde, Y. Dunkel, I. Lopez-Sanchez, Y. Pavlova, A. Marivin, J. Barbazán, F. Murray, U. Nitsche, K.-P. Janssen, K. Willert, A. Goel, M. Abal, M. Garcia-Marcos, P. Ghosh, Daple is a novel non-receptor GEF required for trimeric G protein activation in Wnt signaling. *eLife* **4**, e07091 (2015).
3. P. Ghosh, A. O. Beas, S. J. Bornheimer, M. Garcia-Marcos, E. P. Forry, C. Johannson, J. Ear, B. H. Jung, B. Cabrera, J. M. Carethers, M. G. Farquhar, A Gαi–GIV Molecular Complex Binds Epidermal Growth Factor Receptor and Determines Whether Cells Migrate or Proliferate. *Mol Biol Cell* **21**, 2338–2354 (2010).
4. M. Garcia-Marcos, Heterotrimeric G protein signaling without GPCRs: The *Gα-binding-and-activating* (GBA) motif. *Journal of Biological Chemistry* **300**, 105756 (2024).
5. **A. A. Kendrick**, A. M. Dickey, W. B. Redwine, P. T. Tran, L. P. Vaites, M. Dzieciatkowska, J. W. Harper, S. L. Reck-Peterson, Hook3 is a scaffold for the opposite-polarity microtubule-based motors cytoplasmic dynein-1 and KIF1C. *J Cell Biol* **218**, 2982–3001 (2019).
6. J. R. Christensen*, **A. A. Kendrick***, J. B. Truong, A. Aguilar-Maldonado, V. Adani, M. Dzieciatkowska, S. L. Reck-Peterson, Cytoplasmic dynein-1 cargo diversity is mediated by the combinatorial assembly of FTS–Hook–FHIP complexes. *eLife* **10**, e74538 (2021). *co-first author

AGNIESZKA (AGA) KENDRICK, Ph.D.

Assistant Professor
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Professional positions

Assistant Professor | Salk Institute for Biological Studies 2023 – present

Education and Training

University of California San Diego and Howard Hughes Medical Institute | Department of Cellular and Molecular Medicine | Postdoctoral Fellow, Advisor: Samara Reck-Peterson, Ph.D. 2016 – 2023

University of Colorado Denver | Ph.D., Structural Biology and Biochemistry 2016
Ph.D. Graduate Student, Advisor: Elan Z. Eisenmesser, Ph.D.

University of Colorado Denver | M.S., Chemistry 2010
M.S. Graduate Student, Advisors: Karen R. Jonscher, Ph.D. and Douglas F. Dyckes, Ph.D.

University of Wroclaw | B.S., Chemistry 2005

Fellowships and Grants

American Cancer Society Postdoctoral Fellowship 2018 – 2021
NIH F32 Ruth L. Kirschstein Postdoctoral Fellowship - NIGMS 2018
NIH F31 Ruth L. Kirschstein Predoctoral Fellowship - NCI 2013 – 2016

Honors and Awards

Leading Edge Symposium Fellow 2020
Biophysical Society Travel Award 2016
University of Colorado Graduate School Student Research Excellence Award 2015
C. Werner and Kitty Hirs University of Colorado Graduate School Student Travel Award 2014 – 2016
Protein Society Travel Award 2014
Colorado Biological Mass Spectrometry Society Poster Award 2009
American Society for Biomolecular Facilities Student/Post-Doc Poster Award 2009
University of Colorado Denver Mike Milash Teaching Assistant Award 2009

Equity, Diversity, and Inclusion

DISCOVER – Diverse Inclusive Scientific Community Offering a Vision for an Ecosystem Reimagined program reviewer | The Salk Institute 2024
Leading Edge Panel co-organizer: "Parenting and Family life in Academia" 2022
Work life (im)balance workshop | American Cancer Society TheoryLab and Apple podcast 2021
Elementary School Science Presentations | Highline Academy Charter School 2010 – 2013
Chemistry tutor to Afghan and Libyan refugees 2010 – 2014

Professional Training

COMPASS | NIH-funded professional development and mentorship course | ten 4-hour/week sessions and 6-month support program 2023

Salk Faculty & Mentors Training series | two 3-hour sessions 2023

Professional Service

ASCB subgroup co-organizer: "Not just Cellular railroads: microtubules as cargoes and signaling centers"	2021
Chair of Structural Biology and Biochemistry Program Student Committee | University of Colorado Denver	2013 – 2014
Co-Chair of bi-annual symposium: Translating Structural Biology to Medicine | University of Colorado Denver	2013

Publications

***co-first author, #co-corresponding author**

Research

1. Karasmanis EP*****, Reimer JM*, **Kendrick AA***, Kendrick HVN, Rodriguez JA, Truong JB, Lahiri I, Reck-Peterson SL, Leschziner AE. Lis1 relieves cytoplasmic dynein-1 autoinhibition by acting as a molecular wedge. *Nat Struct Mol Biol.* 2023 Sep; 30(9):10:1357-1364.
2. Christensen JR*****, **Kendrick AA***, Truong JB, Aquilar-Maldonado A, Adani V, Dzieciatkowska M, Reck-Peterson SL. Cytoplasmic dynein-1 cargo diversity is mediated by the combinatorial assembly of FTS-Hook-FHIP complexes. eLife. 2021 Dec 9;10:e74538.
3. **Kendrick AA**, Dickey AM, Redwine WB, Tran PT, Pontano Vaites L, Dzieciatkowska M, Harper JW, Reck-Peterson SL. Hook3 is a scaffold for the opposite-polarity microtubule-based motors cytoplasmic dynein-1 and KIF1C. *J Cell Bio*. 2019; 218(9):2982-3001. F1000 recommended: Kapitein L: F1000Prime, 30 Jul 2019; 10.3410/f.736211626.793563078
4. **Kendrick AA**, Schafer J, Dzieciatkowska M, Nemkov T, D'allessandro A, Neelakantan, D, Ford HL, Pearson CG, Weekes CD, Hansen KC, Eisenmesser EZ. CD147: a small molecule transporter ancillary protein at the crossroad of multiple hallmarks of cancer and metabolic reprogramming. *Oncotarget*. 2017; 8(4): 6742-6762.
5. Ying-Chi C, Rahkola JT, **Kendrick AA,** Holliday MJ, Janoff EN, Eisenmesser EZ. *Streptococcus pneumoniae* IgA1 protease: A metalloprotease that can catalyze in a split manner. *Protein Sci*. 2016; 26(3): 600-610.
6. Saeedi BJ, Kao DJ, Kiitsenberg DA, Dobrinskih E, Schwisow KD, Masterson JC. **Kendrick AA**, Kelly CJ, Bayless AJ, Kominsky DJ, Campbell EL, Kuhn KA, Furuta GT, Colgan SP, Glover LE. HIF-dependent regulation of claudin-1 is central to intestinal epithelial tight junction integrity. *Mol Bio Cell*. 2015; 26(12): 2252-62.
7. **Kendrick AA,** Holliday MJ, Isern NG, Zhang F, Camilloni C, Huynh C, Vendruscolo M, Armstrong G, Eisenmesser EZ. The dynamics of interleukin-8 and its interaction with human CXC receptor I peptide. *Protein Sci*. 2014; 23(4): 464-80.
8. Glover LE, Bowers BE, Saeedi B, Ehrentraut SF, Campbell EL, Bayless AJ, Dobrinskikh E, **Kendrick AA**, Kelly CJ, Burgess A, Miller L, Kominsky DJ, Jedlicka P, Colgan SP. Control of creatine metabolism by HIF is an endogenous mechanism of barrier regulation in colitis. *Proc Natl Acad Sci*. 2013; 110(49): 19820-5.
9. Redzic JS, **Kendrick AA,** Bahmed K, Dahl KD, Pearson CG, Robinson WA, Robinson SE, Graner MW, Eisenmesser EZ. Extracellular vesicles secreted from cancer cell lines stimulate secretion of MMP-9, IL-6, TGF-β1 and EMMPRIN. *PLoS One.* 2013; 8(8): e71225.
10. **Kendrick AA***, Choudhury M*, Rahman SM, McCurdy CE, Friederich M, Van Hove JL, Watson PA, Birdsey N, Bao J, Gius D, Sack MN, Jing E, Kahn CR, Friedman JE, Jonscher KR. Fatty liver is associated with reduced SIRT3 activity and mitochondrial protein hyperacetylation*. Biochem J.* 2011; 433(3): 505-14. *Biochem J* most cited paper of the year (2011).

Commentary

1. **Kendrick AA**[#] and Christensen JR[#]. Bidirectional lysosome transport: a balancing act between ARL8 effectors. *Nat Commun.* 2022; 13, 5261.
2. Humpries BA, Hwang PY, **Kendrick AA**, Kulkarni RP, Pozzar RA, San Martin R. Overstretched and overlooked: solving challenges faced by early-career investigators after the pandemic. *Trends in Cancer.* 2021 Oct;7(10):879-882.

Manuscripts in preparation

Kendrick AA, Leschziner EM, and Reck-Peterson SL. CryoEM captures snapshots of dynein's activation pathway. – I investigated how dynein's mechanochemical cycle is regulated by its regulator, Lis1. Using time-resolved CryoEM I identified multiple conformations of dynein bound to Lis1 or dynein alone (16 unique structures, including 7 unique structures from one dataset), allowing me to map the different steps of dynein's activation in real time. My data shows that Lis1 alters dynein's mechanochemical cycle by transitioning dynein into an activated state more efficiently. I propose a new model for how Lis1 relieves dynein's autoinhibition and promotes conformations that are compatible with motility.

Independent Reviewer

Nature Communications
Journal of Cell Biology

Invited Talks

Molecular Mechanisms of Motors Driving Cellular Movements | 2024 Gordon Research Conference	2024
Cell Biology & Physiology Seminar| Davis, CA	2023
UC Davis Biophysics Seminar| Washington University in St. Louis	2023
Biophysical Society | 2023 Annual Meeting	2023
American Society for Cell Biology | 2022 Annual Meeting Microsymposium	2022
American Society for Cell Biology | 2021 Annual Meeting Subgroup	2021
International Dynein Meeting	2021
Leading Edge Symposium	2020, 2022
American Society for Cell Biology | 2018 Annual Meeting Minisymposium	2018
Colorado Biological Mass Spectrometry Society Meeting	2009

Teaching and Mentorship

Teaching

Teaching Assistant | Structural Biology and Biochemistry Graduate Program | University of Colorado Denver	2011 – 2016
Chemistry tutor | Varies Agencies and Institutions in Denver	2008 – 2015
Chemistry Instructor | Pre-Collegiate Outreach Program | University of Colorado Denver	2008
Teaching Assistant | Department of Chemistry | University of Colorado Denver	2007 – 2010

Mentorship

Kendrick Nguyen | Graduate student | Leschziner lab	2022 – present
Joey Truong | Undergraduate student | Reck-Peterson lab	2019 – 2022
Currently: PhD student, UC Davis	
Donte A. Stevens | Graduate student | Reck-Peterson lab	2018 – present
Andrea Dickey | MD/PhD student | Reck-Peterson lab	2018 – 2022
Currently: Finishing MD, UC San Diego	
Vinit Adani | Undergraduate student | Reck-Peterson lab	2018 – 2019
Currently: PhD student, UC Riverside	
Phuoc Tien Tran | Research Assistant | Reck-Peterson lab	2016 – 2018
Currently: PhD student, Harvard University	
Johnathon Shafer | Research Assistant | Eisenmesser lab	2014 – 2016
Currently: PhD student, UC Denver	