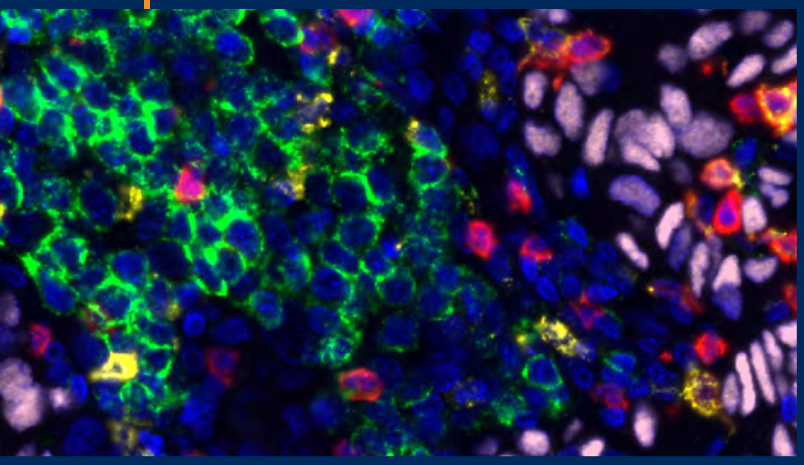
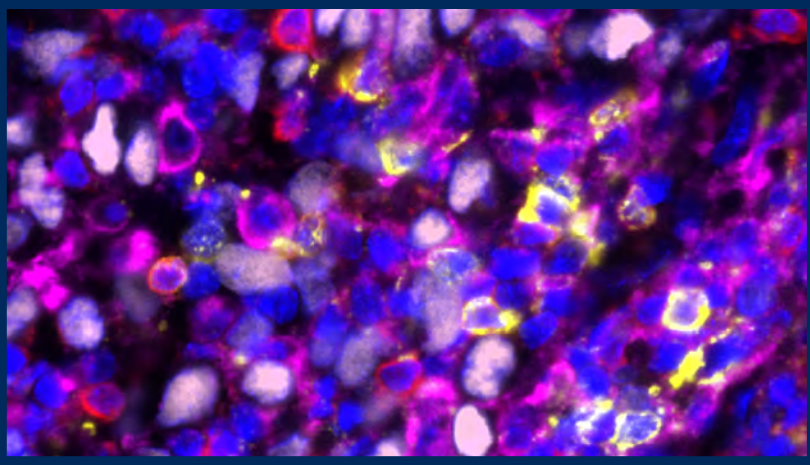
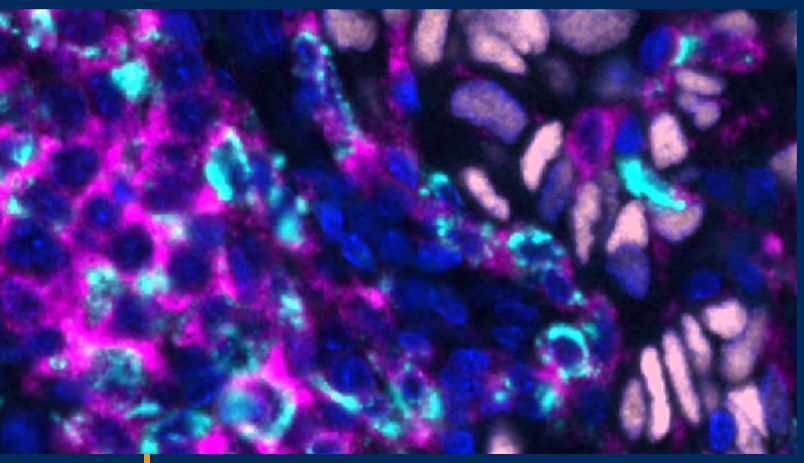
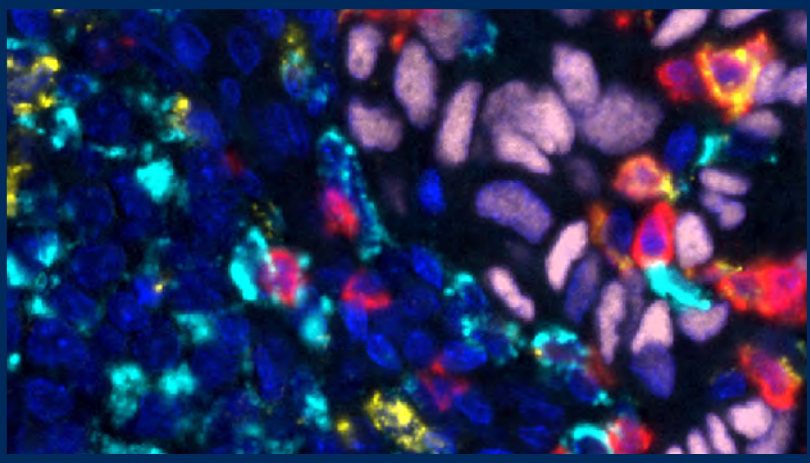
April 2024

Ludwig Link

LUDWIG CANCER RESEARCH

LIFE-CHANGING SCIENCE

Welcome

Unmesh Kher
Editorial Director

Welcome to the spring 2024 Ludwig Link! Regular readers may notice a couple of deviations from the norm in this issue.

First, we've dedicated a little more room than we typically would to one news story (page 8). It's about a three-year team science initiative focused on immunometabolism launched by the Ludwig Institute this year. We thought it deserved a little extra space, not only because of its interesting subject but also for its stress on collaboration across the labs at Institute Branches and at autonomous Ludwig Centers. Second, you'll notice we've mixed things up a bit in our Q&A feature by focusing on Ludwig trainees (page 23). We asked three trainees—two graduate students and a postdoc—to tell us about their lives, science and avocational interests. We had a great time chatting with them, enough to do this again in future Q&As.

What hasn't changed in this issue, happily, is the rich selection of briefs we have for you on recent Ludwig publications. In our Research news section (page 8) you'll learn about pre-existing immune cell networks in tumors that predict whether patients with advanced melanoma are likely to respond to a personalized immunotherapy (see, also, the cover image), how a nutrient in butter might aid immunotherapy, why a timekeeping gene has a contextually variable influence on tumor growth, a safer, more versatile CRISPR technology and its application to make fitter CAR-T cells and much else besides. You will also find in here news about new appointments and promotions at Ludwig Branches (page 7) and the honors lately bestowed on scientists in our community (page 5).

Finally, in our "Ask a scientist" section (page 31) you'll find out how some of your colleagues are using AI in their work and how they think it'll be used in the coming years in cancer research, prevention and care.

Happy reading.

Sincerely,

Unmesh Kher
Editorial Director

On the cover

Micrographs of melanoma tumors depicting networks of activated T cells and myeloid cells or B cells in tumor and stroma, which predict positive patient responses to TIL-ACT immunotherapy. Macrophages are labeled cyan, dendritic cells purple, B cells green and activated PD1+ CD8+ killer T cells red and yellow, or orange in the merged micrograph. Melanoma cells are pink.

STORY ON PAGE 13

In this issue


Johanna Joyce


Sangeeta Bhatia


Laura Attardi


Roeland Nusse


David Pellman

Five Ludwig Cancer Research scientists were elected Fellows of the AACR

Five Ludwig Cancer Research scientists were elected Fellows of the Academy of the American Association for Cancer Research (AACR), class of 2024, in January. The Academy honors scientists whose contributions have fueled significant innovation and progress against cancer. Ludwig Lausanne Member Johanna Joyce was recognized for her contributions to our understanding of the microenvironment of primary and metastatic brain tumors, most notably their immune landscapes. Her work, the Academy noted, has uncovered "effective combination therapies targeting tumor-associated macrophages and microglia in brain metastases." Ludwig MIT's Sangeeta Bhatia was honored for work that is "improving the diagnostic and treatment strategies available for cancer patients" through her pioneering development of micro- and nanotechnologies for use across cancer diagnosis and therapy, drug delivery and tissue regeneration. Ludwig Stanford's Laura Attardi was recognized for her "groundbreaking research dedicated to delineating p53 transcriptional networks, identifying novel p53 target genes critical for tumor suppression, and for characterizing dispensable DNA damage pathways for tumor suppression in the presence of robust p53 activity." Her colleague Roeland Nusse, who is a Virginia and Daniel K. Ludwig Professor of Cancer Research at Stanford University, was honored for his investigations of the Wnt signaling pathway that span the initial discovery and purification of Wnt family proteins to the continued exploration of their biological functions in such processes as tissue regeneration and carcinogenesis—the latter "highlighted by his elucidation of the critical role of Wnt signaling in hepatocellular carcinoma." Ludwig Harvard's David Pellman was recognized for "illustrious contributions to the understanding of cell division functions such as spindle assembly and positioning, asymmetric cell division, and cytokinesis, which, when aberrant, contribute to genomic instability, for developing novel technologies such as single-cell genome sequencing, and for elucidating key regulatory mechanisms in genomic and organismal evolution." Our warmest congratulations to them all.

Jedd Wolchok and Brad Bernstein elected to the NAM

Jedd Wolchok

Brad Bernstein

Our congratulations to Jedd Wolchok, board member of the Ludwig Institute for Cancer Research, and Ludwig Harvard's Bradley Bernstein, who were elected to the National Academy of Medicine (NAM) in October. A clinical oncologist, Jedd is perhaps best known for having led the transformational clinical development of the anti-CTLA-4 drug ipilimumab, the first checkpoint blockade immunotherapy to win regulatory approval. His subsequent contributions include the formulation of criteria to assess patient responses to immunotherapy and groundbreaking studies establishing the enhanced efficacy of combination anti-CTLA-4 and anti-PD-1 immunotherapies for melanoma. A co-director of the Ludwig Collaborative Laboratory at Weill Cornell Medicine, Jedd has also contributed enormously to tumor immunology, and many of his laboratory's findings have led to the creation of diagnostics and therapeutic strategies that are today in various stages of development. Brad, for his part, is an authority on epigenomics. In 2005, he was among the scientists who reported the first large-scale map of human chromatin structure, charting the distribution of a pair of epigenetic tags on two chromosomes and providing an early glimpse of how epigenetics regulates gene expression. A year later, he and his colleagues described for the first time how genes that orchestrate embryonic development are epigenetically tagged to perform their functions—work that upended some fundamental beliefs about how cells control which genes are made available for expression. His subsequent investigations have yielded numerous findings of significance to cancer biology and therapeutics, including the discovery and mechanistic exposition of how non-mutational—purely epigenetic—aberrations in the genome can give rise to cancers. Election to the NAM, as the Academy notes, "recognizes individuals who have demonstrated outstanding professional achievement and commitment to service." We think Brad and Jedd, at the very least, fit that bill.

Chi Van Dang

Chi Van Dang receives AACR-Margaret Foti Award

The American Association for Cancer Research (AACR) announced in mid-February that it would honor Ludwig Institute Scientific Director Chi Van Dang with the 2024 AACR-Margaret Foti Award for Leadership and Extraordinary Achievements in Cancer Research at the Annual Meeting in early April, where Chi will deliver a lecture and officially receive the award. The eponymous award, established in 2007 in honor of the CEO of the AACR, is given every year to a person whose work and leadership have had an enduring impact on the field. A physician-scientist, Chi helped pioneer the modern study of cancer metabolism through his exhaustive study of how the oncogene Myc orchestrates metabolic changes essential to cancer cell growth and survival. More recently, Chi and his colleagues have shown how Myc indirectly disrupts cellular circadian rhythms, linking one fundamental trait of the cancer cell—a disordered metabolism—to another: its broken clock. In issuing the award to Chi, the AACR also noted his many professional contributions as a mentor and scientific leader. These include his role as scientific director of the Ludwig Institute; his former position as Editor-in-Chief of the AACR journal Cancer Research and leadership of numerous committees and advisory groups of the organization; his chairmanship of the National Cancer Institute's Board of Scientific Advisors, and his membership on the Blue Ribbon Panel of the U.S. Cancer Moonshot initiative.

Immunologist Lydia Lynch appointed member of Ludwig Princeton

Ludwig Princeton welcomed immunologist Lydia Lynch to the Branch as a full member in January. A native of Ireland who earned her PhD in 2008 from University College Dublin, Lydia came to the Princeton Branch from Brigham and Women's Hospital and Harvard Medical School, where she was an associate professor of medicine. Her lab, which explores the interplay of systemic and cellular metabolic processes with the immune system, has made landmark contributions to models of how obesity and diet influence immune regulation and anti-tumor immunity, and how immune cells in turn shape systemic metabolism. Lydia was among the first to describe the mechanisms by which changes in systemic metabolism brought about by altered diet affect the metabolism of immune cells in humans and mice, and how obesity compromises immune surveillance and responses to cancer. Lydia also discovered about a dozen years ago a type of immune cell found in fat, the "adipose iNKT cell", that has profound effects on metabolism. She has since detailed the mechanisms by which this unusual class of cells regulates the function of fat cells and body weight. Lydia has recently received major grants to develop a novel innate T cell-based immunotherapy for cancer. In addition to her membership of Ludwig Princeton, Lydia has been appointed full professor in the Department of Molecular Biology at Princeton University.

Lydia Lynch

Michal Bassani-Sternberg is now an assistant member of the Ludwig Institute

Michal Bassani-Sternberg was named an Assistant Member of the Ludwig Institute for Cancer Research, Lausanne Branch, in January. Michal, who the Link interviewed a couple of issues ago, helped pioneer the field of immunopeptidomics—the use of mass spectrometry and computational analysis to predict which antigens out of the thousands proffered to the immune system's T cells are most likely to provoke effective immune responses (see research brief on Page 20). Her work is contributing significantly to both basic tumor immunology and the application of that science to cancer therapy. In their basic research, Michal and her colleagues have had considerable success elucidating the rules that make any given antigen immunogenic. More recently, they have sought to elucidate how those rules play out in the context of the immune microenvironment of the tumor, and how that affects immune responses. On the clinical front, Michal's team has developed a unique and rather complex high-throughput proteogenomics pipeline that can identify in a patient's tumor the various antigens that are likely to be both presented to T cells of the immune system and to be immunogenic. Her technologies are being applied to the development of personalized cancer vaccines and adoptive T cell therapies under development at Ludwig Lausanne and at the Center of Experimental Therapeutics at the University of Lausanne.

Michal Bassani-Sternberg

Exploring the interface of diet, metabolism and anti-tumor immunity

The metabolic weirdness of cancer has fascinated scientists for about a century. In fact, cancer was widely considered a metabolic disease through the 1960s, with the drugs used to treat it often referred to "antimetabolites." With the discovery of the oncogene in 1971, however, cancer came to be seen primarily as a genetic disorder. The pendulum has since swung back somewhat. It's probably safe to say that the current scientific consensus tends to a more nuanced middle ground—that metabolic and genetic dysfunction are both interdependent and of critical importance to carcinogenesis. Indeed, explorations of how the two interact are the stuff of exquisite science today, not least in the context of tumor immunology.

It happens that Ludwig-affiliated labs have made notable contributions

Projects and participating PIs

Matthew Vander Heiden
Ludwig MIT

Stefani Spranger
Ludwig MIT

Benoit Van den Eynde
Ludwig Oxford/Brussels

Edward Pearce
Johns Hopkins University

Eileen White
Ludwig Princeton

Benoit Van den Eynde
Ludwig Oxford/Brussels

Intersection between microbiome, nutrient levels and antitumor immunity

This project will test the hypothesis that the microbiome influences anti-tumor immunity by changing how nutrients in the diet affect those available to T cells in the tumor and in tumor-draining lymph nodes, and that this influences whether or not productive anti-tumor immune responses are generated. The studies will address how dietary amino acids and lipids—and dietary interventions—affect such things as the immunosuppressive properties of the tumor microenvironment, immune cell function, tumor cell metabolism and responses to cancer immunotherapy.

Effects of dietary amino acid perturbations on cancer progression and checkpoint blockade therapy

Altered amino acid metabolism plays a crucial role in cancer initiation, survival and progression, and can drive therapy resistance and regulate immune cell function. This study will examine how dietary perturbations in the availability of four key amino acids—tryptophan, arginine, cysteine and lysine—affect cancer progression and susceptibility to immune checkpoint blockade. The researchers hypothesize that the first two are important primarily because of their role in T cell functionality, while the latter two have distinct and divergent effects on tumor cell biology.

to this arresting body of work. To better tap that reservoir of talent, the Ludwig Institute for Cancer Research launched in January a multi-institutional research initiative focused on diet and immunometabolism—a relatively young field that explores the metabolic crosstalk between tissues and the immune cells that patrol them. Much evidence suggests that the exploitation of this crosstalk is a key mechanism by which tumors disable the anti-cancer immune response. By that same token, a sophisticated understanding of such machinations opens the door to devising new cancer therapies and improving responses to existing ones, most notably immunotherapies.

"We feel our focus on immunometabolism is particularly timely not only because of its translational potential but because new technologies—like single-cell 'omics, multiplex imaging and powerful new approaches for the mass analysis of metabolites—now enable the analysis of complex interactions between metabolism and immune function in unprecedented detail," said Ludwig Institute Deputy Scientific Director Pat Morin.

Over the next few years, the 3-year, $4.2 million Ludwig Immunometabolism Initiative will examine at multiple levels how dietary interventions shape the tumor

Continued on next page

Joshua Rabinowitz
Ludwig Princeton

Erika Pearce
Johns Hopkins University

Ping-Chih Ho
Ludwig Lausanne

Marcia Haigis
Ludwig Harvard

Impact of fructose and carbohydrate restriction on anticancer immunity

Preclinical studies suggest that dietary carbohydrate restriction can enhance anticancer therapy. Such restrictions can occur by fasting or ketogenic diet and may work by limiting carbs in general, or by limiting fructose, which can alter the microbiome and is known to support tumor growth. This project will examine the impact of dietary carb restriction, as well as fructose ingestion, on anticancer immunity. It will focus on colon cancer, using animal models and patient biopsies and address basic questions about nutrient utilization in immune cell subtypes *in vivo*.

Investigating the intersection between carbohydrate and fat metabolism in anti-tumor immunity

Obesity is a major risk factor for multiple cancers, yet little is known about the fates and functions of lipids and their metabolites in antitumor immunity and T cell exhaustion, or how dietary lipid and carbohydrate loads affect these mechanisms. This project will explore the intersection between lipid and glucose metabolism in tumors and immune cells *in vitro* and examine how high fat diets affect the tumor microenvironment in animal models. Another aim is to identify related metabolic pathways that might be targeted for cancer therapy.

Exploring the interface of diet, metabolism and anti-tumor immunity

Continued from previous page

microenvironment (TME) and its immune landscapes, alter cancer progression and modulate responses to therapy, especially immunotherapies. A key requirement for research proposals selected for the program was that they be collaborative, involving partnerships between two Ludwig sites or their host institutions.

"Ludwig is already funding multiple leaders in metabolomics, cancer metabolism and immunometabolism at its Branches and Centers," said Ludwig Institute Scientific Director Chi Van Dang. "We are hopeful that bringing these experts together as teams to answer critical questions in the field will generate actionable insights that will be translated to the clinic."

"I am excited to see the breadth of expertise and the depth of research driven by the funded teams," said Ludwig Institute Executive Vice President for Technology Development Jonathan Skipper. "We are poised as an institute to catalyze laboratory research findings with clinical hypotheses and advance these discoveries into clinical development."

As the collaborations progress, said Pat, new researchers may be brought in as needed to support the core teams. These could include, for example, experts in bioinformatics or machine learning to aid data analysis. It would also be wonderful, he said, if success in these studies draws funding from external governmental or nongovernmental sources to boost Ludwig's efforts.

Xin Lu

Peter Ratcliffe

How deficiency of a regulator of hypoxia inducible factor favors B cell lymphomas

Many tumors depend on the hypoxia inducible factor (HIF) for their survival, and several drugs that inhibit HIF signaling have been approved for cancer therapy. A study published in *PNAS* in February, led by Ludwig Oxford alumnus Jingyi Ma, Director Xin Lu and Distinguished Scholar Peter Ratcliffe explored how a well-characterized cellular regulator of HIF, factor inhibiting HIF (FIH), influences tumor growth. The researchers reported that both copies of the FIH gene are required to prevent the development of spontaneous mouse B cell lymphomas, especially pulmonary B cell lymphoma, during the aging process. FIH deficiency alters the immune composition in aged mice and induces a tumor-promoting immune microenvironment through the manipulation of myeloid cell function: FIH-defective myeloid cells acquire tumor supportive properties in response to environmental cues from cancer cells or cancer-associated inflammation. These properties include enhanced migration into tumors and higher expression of the enzyme arginase, which breaks down arginine, an amino acid essential to T cell activation. Increased levels of arginase reduce T cell proliferation and promote tumor growth. Notably, the FIH-deficient environment could influence the behavior and growth of injected cancer cells in what can be called an "outside-in" model—highlighting the importance of the external microenvironment on tumor growth.

Deficiency of factor-inhibiting HIF creates a tumor-promoting immune microenvironment | *PNAS*, 2024 February 29

A math model of how interstitial fluid flow influences cell movement in tumors

Leaky blood vessels and cell proliferation within tumors contribute to increased interstitial fluid flow, a phenomenon that is thought to influence the movement of cancer cells and metastasis. Researchers co-led by Ludwig Oxford's Helen Byrne examined, via mathematical modeling, how the mechanical and chemical landscape around cells might influence the direction of their migration. The interstitial flow creates two opposing forces. One, which the researchers call tensotaxis, draws the cells "upstream", or against the interstitial flow, as a consequence of their cytoskeletal responses to changes in external pressure. The other, chemotaxis, pulls the cells in the direction of the flow, or "downstream", in response to flow-induced gradients of chemoattractants that are produced by the moving cell itself and swept along with the interstitial flow. The latter mechanism has been observed in tumors, with cancer cells producing the ligand CCL21 and migrating downstream as the chemoattractant binds their own CCR7 receptors. Studies have also shown that changes in interstitial flow conditions and local cell density can affect these competing stimuli and alter the direction of cell migration. Helen and her colleagues reported in a February paper in the *Biophysical Journal* a mathematical model that predicts how the balance between these competing mechanisms changes over time and determined the conditions under which cells' transitions from upstream to downstream migration occur.

Helen Byrne

Using a probabilistic approach to derive a two-phase model of flow-induced cell migration | *Biophysical Journal*, 2024 February 26

An RNA-editing platform, used for metabolic engineering, boosts CAR-T performance

Researchers co-led by Ludwig Stanford's Crystal Mackall reported in a February publication in *Cell* their development of a potentially safer, more versatile CRISPR editing platform named MEGA (for Multiplexed Effector Guide Arrays) and demonstrated in preclinical studies that it holds the potential to dramatically improve the efficacy of CAR-T cell immunotherapies. The platform employs CRISPR-Cas13d—which targets RNA, not DNA—to edit multiple transcripts of genes at once while nixing the ever-present risk associated with erroneous, or "off-target", edits made to genomic DNA, which can themselves theoretically seed cancer. The researchers employed MEGA in a screen in which they knocked down 6,400 paired combinations of genes in cultured T cells to find novel paired genes that could be targeted to improve CAR-T cell function. They also engineered the RNA-editing system to sport an on/off switch, linking its programmable execution of multiplexed CAR-T cell engineering to the presence of an FDA-approved antibiotic. Finally, Crystal and her colleagues demonstrated how MEGA could be used to improve the metabolic fitness of CAR-T cells, showing that their engineering of the cells altered the way in which they generate energy, switching from glycolysis to oxidative phosphorylation, which greatly improved their persistence within tumors. This boosted their proliferative capacity by an order of magnitude and improved tumor suppression to a similar degree in a mouse model of cancer.

Crystal Mackall

A versatile CRISPR-Cas13d platform for multiplexed transcriptomic regulation and metabolic engineering in primary human T cells *Cell*, 2024 February 21

Colin Goding

DNA damage remodels the MITF interactome to increase melanoma genomic instability | *Genes & Development*, 2024 February 5

Study uncovers an unexpected mechanism by which a key oncogene drives melanoma

A study led by Ludwig Oxford's Colin Goding and alumnus Romuald Binet showed that the microphthalmia-associated transcription factor MITF shapes melanocyte responses to DNA damage in an unexpected way. The researchers reported in a February paper in *Genes & Development* that DNA-damaging agents induce MITF phosphorylation on serine 325 and alter the protein's interaction with other proteins. MITF then dissociates from its usual partners involved in gene expression and now interacts with the MRN protein complex, which repairs DNA and triggers cell cycle arrest. This stalls the DNA repair pathway, contributing to genomic instability. In agreement with this, high MITF levels are associated with increased single-nucleotide mutations and copy number abnormalities in the genomes of cancerous melanocytes. Notably, a mutation in MITF (E381K) that is known to predispose people to melanoma recapitulates the observed effects of MITF phosphorylation—suggesting a mechanism for the cancer risk associated with the mutation. The authors speculate that under physiological conditions, the penalty imposed by potential genome instability caused by reduced MRN complex function when it is bound by MITF would be offset by increased melanocyte proliferation and their ultimate differentiation that would protect against subsequent DNA damage.

Christopher Douville

Machine learning to detect the SINEs of cancer | *Science Translational Medicine*, 2024 January 24

A biomarker of repetitive DNA elements could improve analysis of liquid biopsies

Researchers co-led by Ludwig Johns Hopkins' Christopher Douville described in a *Science Translational Medicine* paper in January a machine learning approach to profile Alu elements—short, repetitive DNA sequences that jump about the genome and account for about 10% of our genomic sequences—for cancer detection. Alu elements have long had potential as cancer biomarkers but their use for that purpose has been limited by difficulties associated with their analysis that stem from their repetitive nature. Named Alu Profile Learning Using Sequencing (A-PLUS), the method developed by the Ludwig Johns Hopkins team was applied to 7615 plasma samples from 5178 individuals: 2073 of them patients with solid cancers and the remainder controls without a diagnosis of cancer. A-PLUS alone provided a sensitivity of 40.5% across 11 different cancer types in the validation cohort, at a specificity of 98.5%. Combining A-PLUS with DNA sequence-based detection of aneuploidy—an abnormal number of chromosomes common to cancer—and eight common protein biomarkers detected 51% of the cancers at 98.9% specificity. Christopher and his colleagues found that the power of their approach stemmed in large part from a single feature: the global reduction of a subtype of Alu elements—AluS—in the circulating DNA shed by solid tumors. The study suggests the evaluation of Alu elements could improve the performance of several different analytical methods applied to liquid biopsies.

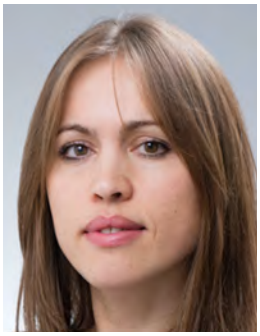
Immune cell networks in tumors predict response to an immune cell therapy for melanoma

Researchers led by Ludwig Lausanne's David Barras, Eleonora Ghisoni, Johanna Chiffelle, Denarda Dangaj Laniti and Branch Director George Coukos reported in a *Science Immunology* paper in February that pre-existing immune cell networks in tumors predict whether patients with advanced melanoma are likely to respond to a personalized immunotherapy known as TIL-ACT. In TIL-ACT, tumor infiltrating lymphocytes (TILs) that kill cancer cells are isolated from tumors, grown in the lab and infused into patients for therapy. The researchers collected tumor samples from patients enrolled in a small trial at Lausanne before TIL-ACT therapy started and then at various time points thereafter. Tumors that responded best to TIL-ACT were those that were most riddled with mutations and harbored killer T cells in states with a potential for intense anti-tumor activation. The immune cell networks the researchers identified consist of killer T cells in close association with activated dendritic cells and macrophages. Successful TIL-ACT therapy further expanded and activated these immune cell networks. Macrophages additionally expressed a molecule named CXCL9 that likely bolsters stimulatory interactions with T cells. The study described biomarkers that, with further vetting, could help clinicians select patients for TIL-ACT—which was approved for treating melanoma for the first time in the U.S. in February. The findings could also inform interventions to broaden the pool of patients who benefit from the therapy.

David Barras

Eleonora Ghisoni

Johanna Chiffelle



Denarda Dangaj Laniti

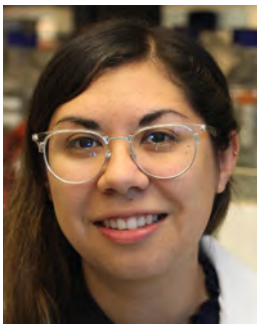
George Coukos

Response to tumor-infiltrating lymphocyte adoptive therapy is associated with preexisting CD8+ T-myeloid cell networks in melanoma | *Science Immunology*, 2024 February 2

Study suggests co-targeting of G-MDSCs and macrophages to treat melanoma

Colony-stimulating factor-1 receptor (CSF1R) inhibitors, which deplete a subset of immune cells that support cancer growth known as tumor-associated macrophages (TAMs), have had limited success in patients. Ludwig Stanford's Allison Banuelos, Allison Zhang and Director Irv Weissman explored the causes of such failure using a mouse model in which the CSF1R could be inducibly deleted. They reported in a January paper in *PNAS* that systemic and intratumoral increases in numbers of granulocytic myeloid-derived suppressor cells (G-MDSCs) contribute significantly to resistance to CSF1R inhibitors in a mouse model by undermining the ability of macrophages to gobble up cancer cells and debris (phagocytosis), which is a key function of the antitumor version of these cells. Targeting G-MDSCs by CXCR2 inhibition improved the ability of macrophages to attack cancer cells. Combining CXCR2 inhibition with CD47 blockade—a therapy developed by Irv's group, now in clinical development, that silences a "don't eat me" signal transmitted by cancer cells to macrophages—boosted macrophage phagocytosis and delayed tumor progression in a mouse model of melanoma. This indicates G-MDSCs play a central role in melanoma's suppression of immune responses. The researchers suggest combining CD47 and CXCR2 inhibition might be a potent strategy to boost the efficacy of CD47 blockade for cancer therapy.

CXCR2 inhibition in G-MDSCs enhances CD47 blockade for melanoma tumor cell clearance | *PNAS*, 2024 January 23



Allison Banuelos

Allison Zhang

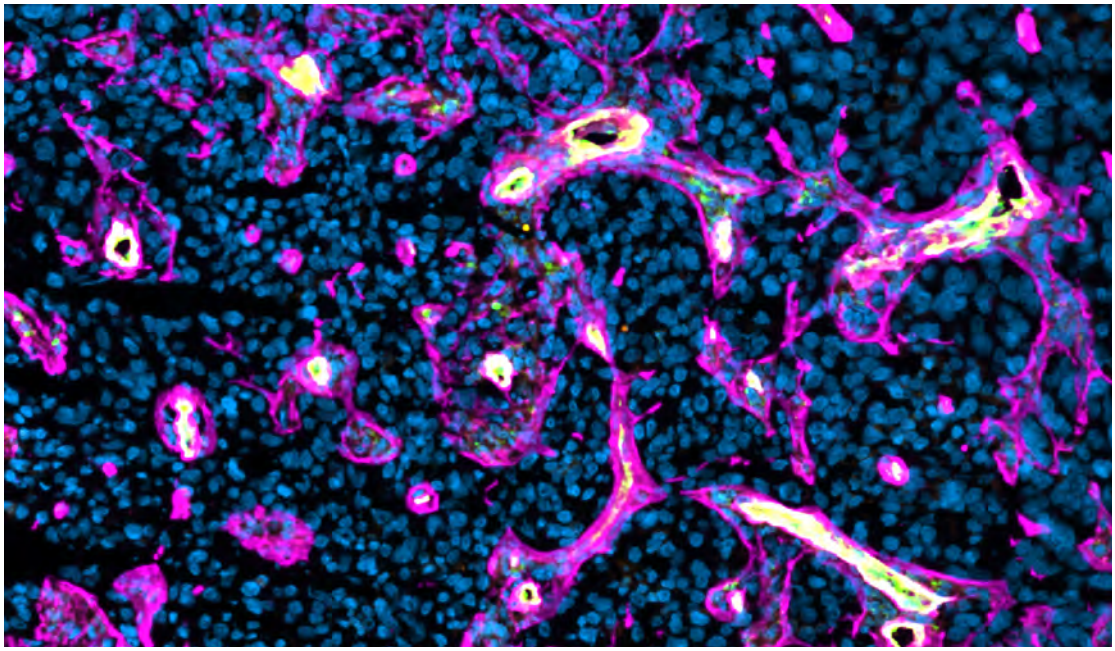
Irv Weissman

Joshua Rabinowitz

The balance of glycine and serine in mice is maintained exclusively by their consumption

A key player in metabolism, the folate-dependent enzyme serine hydroxymethyltransferase (SHMT) reversibly converts the amino acid serine into another amino acid, glycine, releasing a tetrahydrofolate-bound one-carbon unit. This process plays a critical role in development, the immune system and in cancer: SHMT2 is a driver of both cancer cell proliferation and T cell activation. A team led by Ludwig Princeton Director Joshua Rabinowitz reported in *Cell Metabolism* in January that the biosynthesis of serine and glycine is not sensitive to dietary levels of these amino acids in mice and that their homeostasis—or appropriate systemic balance—is maintained by glycine consumption in the liver, where the folate pathway runs in reverse to generate serine. Targeting this process in mice by the pharmacological inhibition of SHMT1/2 or by the deletion of these enzymes in the liver led to an 8-fold surge in systemic levels of glycine. Josh and his colleagues showed that when there is an insufficient supply of glycine or serine, mice adjust by modulating their consumption rather than stepping up their production. Isotope labeling revealed that the serine produced from glycine by the liver is converted into pyruvate and burned to produce energy via the TCA cycle, a core process of normal cellular respiration. The findings are of considerable relevance to cancer therapy as serine/glycine-free diets have anticancer activity in mice and are currently being tested in humans.

Glycine homeostasis requires reverse SHMT flux | *Cell Metabolism*, 2024 January 2



Micrograph of a brain metastasis tissue sample in which endothelial and mural cells of blood vessels are labeled in green and orange, respectively, surrounded by Collagen-V labeled in pink.

Analysis of brain tumor blood vessels yields a candidate therapy and a platform to reveal many more

Ludwig Lausanne's Leire Bejarano and Johanna Joyce presented in a January paper in *Cancer Cell* a granular portrait of how the cellular and molecular components of the blood vessels of lung-, melanoma-, and breast cancer-brain metastases differ from those of healthy brain tissue and how they help shape the tumor microenvironment. The researchers conducted a deep analysis of the cells that form the blood-brain barrier in noncancerous brain tissue and metastatic brain tumor samples freshly isolated from patients. They then focused on endothelial cells (ECs), which line the inner vascular surface, and mural cells—pericytes and smooth muscle cells—that swaddle and stabilize blood vessels. Their analysis revealed multiple aberrations in tumor vessels, including impaired intercellular junctions and adhesiveness of ECs and mural cells that were recapitulated in mouse models of brain metastasis. The murine studies revealed that mural cells and ECs regulate immune cell trafficking into brain metastases. The researchers then focused on CD276, which was highly expressed in ECs and mural cells. CD276 supports immune evasion by cancer cells and is associated with poor patient outcomes. Leire, Johanna and their colleagues showed that antibodies targeted to CD276 extended survival in a mouse brain metastasis model. The researchers also highlighted the utility of their integrated cross-species platform for the identification of additional therapeutic targets in the vasculature of brain metastases.

Leire Bejarano

Johanna Joyce

Interrogation of endothelial and mural cells in brain metastasis reveals key immune-regulatory mechanisms | *Cancer Cell*, 2024 January 18

A central timekeeping gene has a contextually variable influence on tumor growth

A study led by Ludwig Institute Scientific Director Chi Van Dang and Research Associate Xue Zhang found that the circadian clock, rather than being invariably tumor suppressive, has a contextually variable role in cancer. The researchers reported in a January paper in *Nature Communications* that loss of Bmal1, a master regulator of the cellular clock, dampens rather than accelerates the growth of melanoma tumors in mice because it compromises the activity of HIF1, which coordinates cellular adaptations to the hypoxic conditions. Chi, Xue and colleagues also showed that the effects of over-expressed Bmal1 on melanoma growth had nothing to do with its primary role as a regulator of gene expression. Rather, in melanoma cells ectopic Bmal1 binds and sequesters a protein involved in cell motility, MYH9, that is thought to be a tumor suppressor. The researchers detailed MYH9's mechanism of tumor suppression and showed that Bmal1's sequestration of MYH9 reverts melanoma cells into an immature "mesenchymal" state associated with resistance to immunotherapy. Melanoma cells expressing high levels of Bmal1 form tumors in mice that are poorly infiltrated with anti-cancer immune cells and tend to house immunosuppressive cells instead. The findings show Bmal1 drives tumor growth and immunotherapy resistance in melanoma and suggest its effect on tumorigenesis likely varies between cancer types.

Cell state dependent effects of Bmal1 on melanoma immunity and tumorigenicity | *Nature Communications*, 2024 January 20

Chi Van Dang

Xue Zhang

Constantine Mitsiades

A trove of data on cancer cell vulnerability to NK cell killing

Researchers co-led by Ludwig Harvard's Constantine Mitsiades explored the genetic determinants and mechanisms of cancer cell susceptibility and resistance to natural killer (NK) cell targeting using single-cell and genome-scale functional genomics screens of interacting NK and blood cancer cells. They also profiled the sensitivity of multiple blood cancer cell lines to NK cell targeting and evaluated mechanisms of sensitivity to NK killing and escape from such targeting via CRISPR gene editing screens. By integrating these data and patient genomic profiles, Constantine and his colleagues generated a comprehensive portrait of functionally validated molecular mechanisms that influence how NK cells recognize and kill malignant hematopoietic cells, and how those cells escape targeting. They reported in a December paper in *Immunity* that the interaction of NK and cancer cells activates the former and induces in both transcriptional states—including interferon-related gene expression—that depend on the type and molecular features of the cancer cell. The study identified a panoply of genes regulating sensitivity and resistance to NK cell killing, including adhesion-related glycoproteins, protein fucosylation genes and transcriptional regulators. The publicly available data, which include gene targets that induce susceptibility to NK cell killing, hold clues to the design of novel immunotherapies.

Single-cell functional genomics reveals determinants of sensitivity and resistance to natural killer cells in blood cancers | *Immunity*, 2023 December 12

BAMBI's degradation compromises radiotherapy

Radiotherapy kills cancer cells both directly and through the instigation of anti-tumor immune responses, though tumors tend to develop resistance to the latter. Researchers led by Ludwig Chicago's Liangliang Wang, Hua Laura Liang and Director Ralph Weichselbaum reported in a December paper in the *Journal of Clinical Investigation* a previously unknown mechanism by which such immune evasion occurs. It involves immunosuppressive myeloid-derived suppressor cells (MDSCs), which are immune cells that infiltrate tumors. The researchers found that in both mice and humans, ionizing radiation specifically dampens in MDSCs the expression of a protein named BAMBI. This protein is known to interfere with signaling by another protein, TGF-ß, which drives tumor progression. The researchers discovered that the m6A RNA-binding protein YTHDF2, which is also activated in MDSCs by ionizing radiation, directly binds and degrades transcripts of the BAMBI gene to silence its expression. Overexpressing BAMBI in MDSCs compromised their infiltration of tumors and suppression of immune responses. High expression of the protein in patients is associated with longer overall survival times for four types of cancer. Further, delivering BAMBI to the tumor microenvironment boosts the effects of radiotherapy and radioimmunotherapy in mouse models of cancer. The researchers suggest their findings support targeting BAMBI for cancer therapy.

Epitranscriptional regulation of TGF-β pseudoreceptor BAMBI by m6 A/YTHDF2 drives extrinsic radioresistance | *Journal of Clinical Investigation*, 2023 December 15

Liangliang Wang

Hua Laura Liang

Ralph Weichselbaum

Chuan He

A butter-borne abettor of immunotherapy

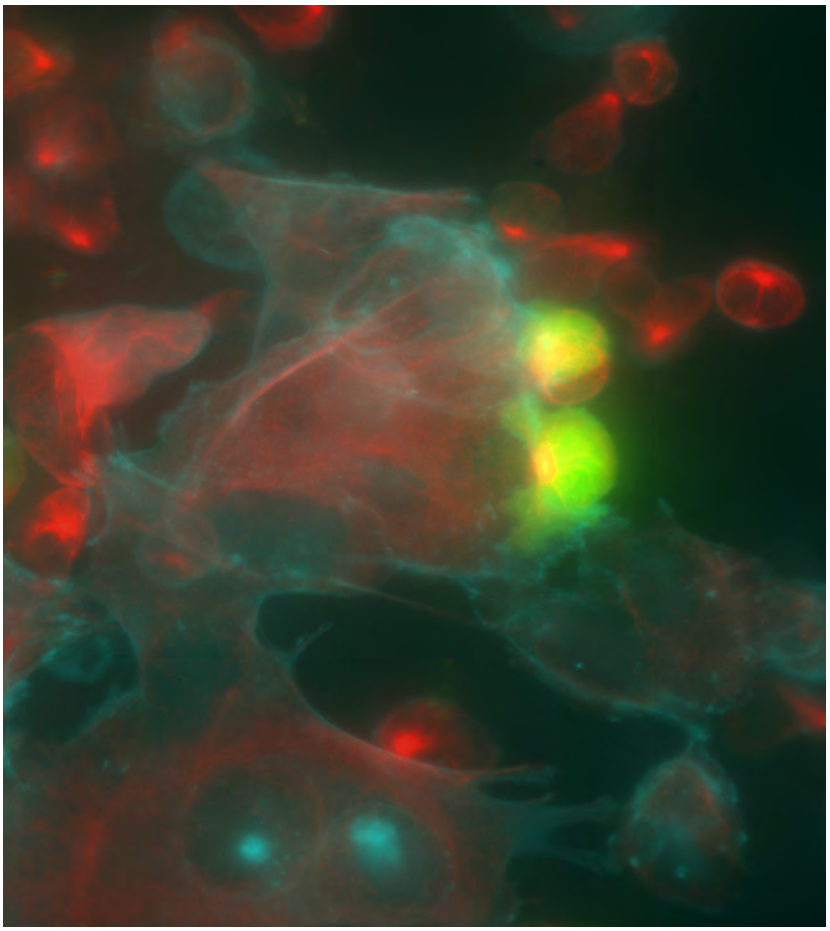
Researchers co-led by Ludwig Chicago's Chuan He reported in a November paper in *Nature* that dietary trans-vaccenic acid (TVA), the predominant trans fatty acid in human milk, directly promotes effector CD8+ T cell function and anti-tumour immunity in animal models. Only about 12%-19% of TVA—which cannot be produced by humans and comes primarily from beef, lamb and dairy products—is converted into a derivative, indicating it has some other activity. Chuan and his colleagues homed in on it when they constructed a blood nutrient library totaling 255 compounds and screened them for their ability to activate CD8+ T cells. TVA came up tops. The researchers found that TVA inactivates the GPR43 receptor on T cells, which is activated by fatty acids produced by gut bacteria. GPR43 silencing switches on signaling through the cAMP–PKA–CREB axis, a cellular signaling pathway that drives T cell survival, differentiation and proliferation. Analysis of blood samples from lymphoma patients showed that those with higher levels of circulating TVA tend to respond better to CAR-T cell immunotherapy. Supplementation with dietary TVA suppressed tumor growth in mouse models of colon cancer and melanoma, and appeared to improve T cell infiltration of tumors. It also notably reduced metastasis in a spontaneous colon cancer model. These effects were lost in mice engineered to lack the GPR43 receptor. The findings suggest TVA could prove to be an effective dietary supplement to elevate human immunity against cancer and improve outcomes of cancer immunotherapy.

Trans-vaccenic acid reprograms CD8+ T cells and anti-tumour immunity | *Nature*, 2023 November 22

Novel CRISPR screen uncovers adaptive resistance mechanism to CAR-T cell therapy

Although CAR-T cell therapies have significantly improved the treatment of B cell malignancies, many patients relapse even after achieving apparently complete responses. Researchers led by Ludwig MIT's Michael Hemann reported in a December paper in *Nature Communications* their exploration of cancer cell-intrinsic mechanisms of resistance to the therapy in a preclinical model of B cell-acute lymphoblastic leukemia (B-ALL) using a novel CRISPR-Cas9 loss-of-function genetic screen. Combining gene expression and in vivo screening data obtained from relapsed B-ALL cells led to the identification of components of the IFNγR/JAK/STAT signaling pathway as key elements of a mechanism of CAR-T therapy resistance. Relapsed murine tumors exhibited heightened activity of this pathway. Functional studies identified a target of this pathway, Qa-1b—the murine homolog of Human leukocyte antigen E (HLA-E)—as essential to the observed resistance mechanism. Functional studies revealed that the mechanism involved cancer cells' simultaneous interaction with both CAR-T cells and natural killer (NK) cells, with Qa-1b binding a receptor on CAR-T cells to inhibit their activity. NK cell depletion in mice abrogated the effect, significantly extending survival following treatment with CAR-T cell therapy. Ditto for antibodies that blocked Qa-1b interaction with its receptor. Michael and his colleagues note that their findings suggest an approach to improving CAR-T therapy for B-ALL that does not require modification of the CAR-T cells themselves.

Leukemia-intrinsic determinants of CAR-T response revealed by iterative in vivo genome-wide CRISPR screening | *Nature Communications*, 2023 December 5



Two CAR-T cells (green) shown here targeting glioblastoma cells.

Michael Hemann

Finding the oncogenic culprit in amplified chromosomal segments that drive cancer

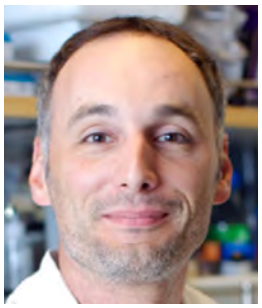
Though copy number gains of chromosomal segments are common in cancer, and known to influence disease progression, discerning the genes within them that drive cancer has proved challenging because these aberrations tend to span large regions of chromosomes. Researchers co-led by Ludwig Stanford's Calvin Kuo analyzed data from The Cancer Genome Atlas and conducted experiments using organoid models to address this challenge. To identify potential oncogenes, the researchers applied computational analysis to detect extreme somatic copy number amplifications that appeared to be coincident with abnormally heightened gene expression, which would suggest the regions contribute to tumor growth and survival. They then validated the "outlier" oncogenes proposed by their analysis by screening them using tissue-specific lentiviral libraries in corresponding organoid tissues modeling esophageal, oral cavity, colon, stomach, pancreatic and lung cancers. The approach allowed Calvin and his colleagues to genetically and pharmacologically evaluate potential targets for therapy once they had zeroed in on oncogenic drivers within the genomic regions encoding extreme copy number amplification. They reported in *Cell Reports* in November their identification of DYRK2 and FGF3 as outlier oncogene candidates in organoids modeling oral and esophageal squamous cancers, respectively, that lacked expression of the p53 tumor suppressor. Calvin and his colleagues also showed that FGF inhibitors dampen the growth of esophageal tumors characterized by FGF3 copy gain.

Calvin Kuo

Functional screening of amplification outlier oncogenes in organoid models of early tumorigenesis
Cell Reports, 2023 November 1

An alternative driver of SCLC metastasis illustrates the cancer's plasticity

Previous studies conducted with mouse models and on patient tumors have implicated the transcription factor NFIB as a primary driver of the growth and metastasis of small cell lung cancer (SCLC), an aggressive cancer that metastasizes readily. A study led by Ludwig Stanford's Julien Sage used conditional gene knockout strategies in genetically engineered mouse models of the cancer to establish NFIB's role in tumor progression and metastasis and its suitability as a drug target for SCLC treatment. They reported in a November paper in *Cancer Research* that while heightened activity of NFIB contributes to tumor progression, the protein is not absolutely required for metastasis. Instead, the researchers identify transcription factors FOXA1 and FOXA2—pioneer transcription factors that can, unlike most transcription factors, interact with inactive regions of chromosomes and activate silent genes—as candidate drivers of SCLC metastasis. Julien and his colleagues point out that this alternative pathway to metastasis in SCLC highlights the notable plasticity of this cancer. Beyond that, the finding identifies additional targets for the treatment of the generally lethal cancer.



Julien Sage

Small cell lung cancer plasticity enables NFIB-independent metastasis | *Cancer Research*, 2023 November 14

A substitute steps up when SWI/SNF chromatin remodeling complex is targeted for therapy

The SWI/SNF complex is a remodeler of chromatin that unspools DNA from the fundamental unit of its histone protein packaging—the nucleosome—to make sequences that initiate and boost gene expression available for recognition by the cellular machinery assigned those tasks. Drugs that target SWI/SNF activity for cancer therapy are now in clinical development, as the complex is mutated in nearly a quarter of all cancers. But relatively little is known about the full spectrum of SWI/SNF gene targets and what the functional effects of its inhibition might be. Ludwig Harvard's Karen Adelman and colleagues used an inhibitor of SWI/SNF activity to address those questions in experiments conducted using multiple cancer cell lines. They reported in a November paper in *Cell* that SWI/SNF inhibition can fall short of its intended effect because an alternative chromatin remodeler, EP400, steps up to compensate for its loss. Inhibiting both remodelers resulted in suppression of cancer cell growth in patient-derived cell lines of acute myeloid leukemia, the pediatric brain cancer diffuse intrinsic pontine glioma, prostate cancer and non-small cell lung cancer. Aside from identifying a new "synthetic lethality" and potential drug target for combination therapy, the findings uncover molecular genomic features that could predict sensitivity to SWI/SNF inhibition.

Global identification of SWI/SNF targets reveals compensation by EP400 | *Cell*, 2023 November 2

Karen Adelman

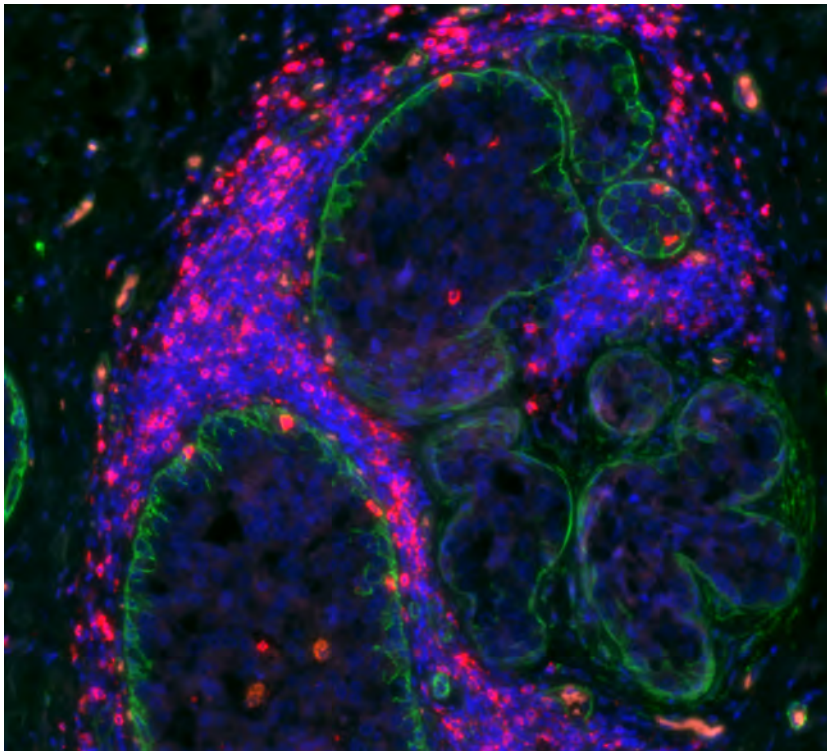
Michal Bassani-Sternberg

Markus Müller

Machine learning method improves neoantigen selection for immunotherapy

Personalized immunotherapies target neoantigens, which are generated by random mutations across the cancer genome. But prioritizing these antigens by their predicted immunogenicity—or ability to provoke effective immune responses—is a major challenge and vital to the optimal design of personalized cancer vaccines, which may only include a small subset of them. Yet methods for such prioritization that use specific criteria—like how likely they are to be presented to and recognized by T cells—have produced little consensus in their rankings or consistency in their performance across datasets. Machine learning methods, which can train algorithms while simultaneously taking into account multiple criteria, have performed much better. Researchers led by Ludwig Lausanne's Michal Bassani-Sternberg and Markus Müller reprocessed data from three large immunogenicity screening assays from 131 cancer patients and applied machine learning to train algorithms to select the most promising neoantigens. Their approach, reported in an October issue of *Immunity*, accurately predicted immunogenicity across datasets and achieved a 30% improvement on the ranking of neoantigens. The algorithm also furnished useful insights on the criteria that best predict neoantigen immunogenicity. Their methods will find good use in the design of personalized immunotherapies at Ludwig Lausanne.

Machine learning methods and harmonized datasets improve immunogenic neoantigen prediction | *Immunity*, 2023 October 9



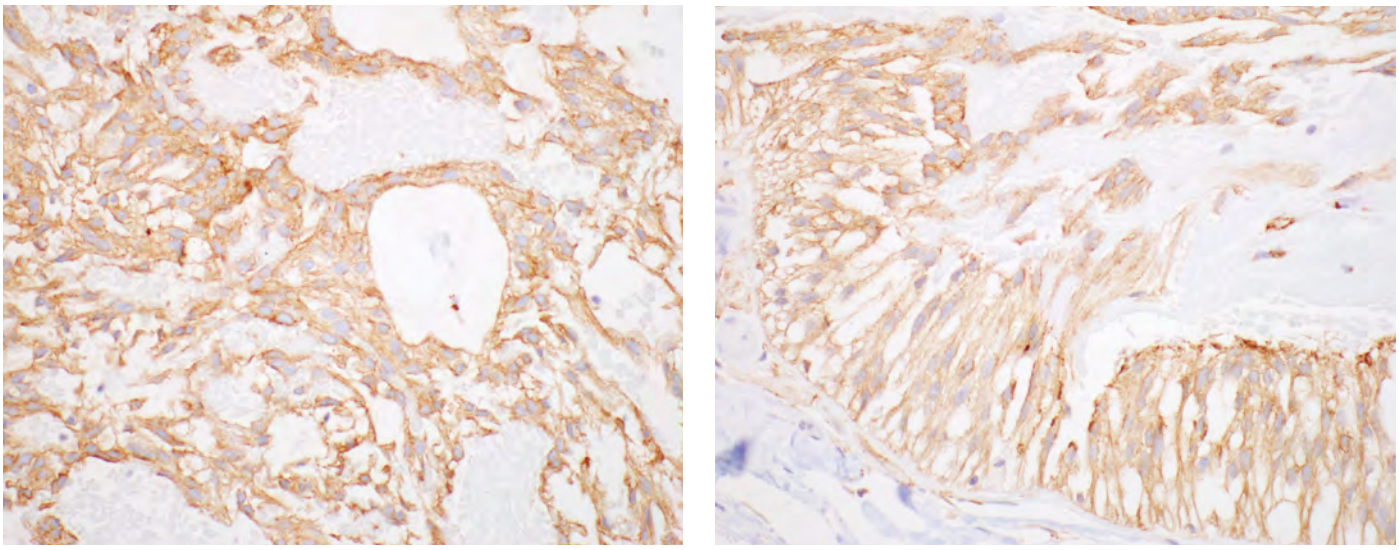
A micrograph showing CD8 T cells (stained red) and smooth muscle actin-positive (green) myoepithelial cells that line the ducts in breast tissue.

T cell receptor clonotype diversity in breast cancer patients at various stages and ages

Researchers led in part by Ludwig Harvard's Kornelia Polyak studied the diversity of the T cell receptor (TCR) clonotypes—a unique nucleotide sequence that arises during the gene rearrangement process for the TCR—in the peripheral blood of 485 breast cancer patients diagnosed with ductal carcinoma *in situ* (DCIS) or *de novo* stage IV disease before or after age 45. The tumor immune landscape in patients changes as they progress through the stages of breast cancer, from ductal hyperplasia to metastatic disease, and Kornelia and her colleagues have previously described increasing immunosuppression and decreasing TCR clonotype diversity in the tumor microenvironment during disease progression. There is, however, also growing evidence to suggest that TCR clonotype diversity of circulating T cells—a quantitative measure of immune health that seems to decrease with age—might similarly influence disease progression and anti-tumor immunity. Kornelia and colleagues reported in *PNAS* in November that such "peripheral" TCR clonotype diversity is lower in older patients regardless of disease stage, and in younger patients with advanced disease compared to those with DCIS. Notably, in older patients, those with DCIS and a higher peripheral clonotype diversity were more likely to suffer a relapse. The findings underscore the importance of peripheral immunity and age in disease progression and the authors suggest peripheral TCR clonotype diversity should be further explored in large clinical studies as a biomarker for disease progression.

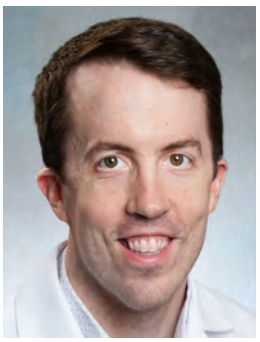
Kornelia Polyak

Peripheral blood TCR clonotype diversity as an age-associated marker of breast cancer progression | *PNAS*, 2023 November 27



HER2 immunohistochemistry of myxopapillary ependymoma, a rare tumor that arises in the lumbar spinal cord that has spread across the leptomeningeal surfaces of the patient's spine. These tumors have no targetable genetic driver, but express HER2 protein (brown color indicates HER2 protein, blue color indicates counterstain showing tumor cell nuclei). The presence of HER2 protein in these tumors offers an opportunity to use FDA approved antibody drug conjugate (ADCs) agents, such as T-DXd, that have shown activity in breast cancer.

Sandro Santagata



Shannon Coy

Many CNS tumors may be vulnerable to existing antibody-drug conjugates

Antibody-drug conjugates (ADCs) enhance the specificity of cytotoxic drugs used for cancer treatment by directing them to cells expressing target antigens, potentially improving their efficacy and reducing their side effects. Many such drugs have in recent years been approved as cancer therapies and, as suggested by a recent study of patients with brain metastases of breast cancer, seem to work even when the targeted antigen is expressed at low levels. Still, the expression of ADC targets is poorly characterized in many central nervous system (CNS) tumors. Researchers led by Ludwig Harvard's Sandro Santagata and Shannon Coy analyzed publicly available RNA-sequencing and proteomic data to evaluate the expression of 14 potential ADC targets that are FDA-approved or under investigation in solid cancers, examining ten different types of CNS tumors. Sandro, Shannon and their colleagues reported in *Neuro-Oncology* in October that most CNS tumors exhibit subtype-specific expression of ADC targets, including several that have been approved by the FDA for other indications. This opens the possibility of personalizing therapy for brain tumors, including rare ones, based on ADC target expression. Of high interest is glioblastoma, which expresses several potential ADC targets, although more work will be needed to find optimal targets for this highly aggressive malignancy. The researchers suggest that their findings support the clinical evaluation of ADCs for the treatment of many CNS tumors.

Systematic characterization of antibody-drug conjugate targets in central nervous system tumors | *Neuro-Oncology,* 2023 October 23

LUDWIG'S TRAINEES

On the front lines

Three early-career researchers—two in grad school, one all set to start his own lab—put down their pipettes to chat with us about themselves, their research and various other matters.

Hannah Fuchs

MD-PhD candidate

Ludwig Oxford, Xin Lu lab

Jenna AbuSalim

MD-PhD candidate

Ludwig Princeton, Joshua Rabinowitz lab

Ángel Álvarez-Prado

Postdoctoral fellow

Ludwig Lausanne, Johanna Joyce lab

Hannah Fuchs

MD-PhD candidate, Ludwig Oxford, Xin Lu laboratory

Tell us a bit about yourself.

I'm originally from Germany, I grew up in Düsseldorf. I learned English in school, but I was never particularly set on leaving Germany. After school I was really torn between whether to do a science degree or study medicine. Then I came across the medicine course in Oxford, which is quite science heavy. You get to do a research project in your undergrad—I'd never heard of that anywhere in Germany. So I did the first four years of med school and then I took a break from that. I've done all of my preclinical training and I've done my first year of my clinical training as well. Then I'm going back to medical school this July. I'm just writing my thesis now.

I feel very fortunate that I've been able to do it in this order because I'd always dreamed of doing a PhD way later down the line, but I didn't realize that was something I could do in med school. I've found it really beneficial, especially working on a fairly clinical project. Even though I hadn't spent that much time in the hospital, it's given me a real appreciation of how clinical day-to-day life works and what practical struggles there might be running trials from a clinical side.

What is the focus of your research?

I work on the checkpoint inhibitor esophageal cancer trial—that's the LUD2015-005 trial. I am investigating what sort of immune cells are present in the cancer and how that determines whether patients, and which patients, respond to treatment. I've worked on two big things. I've tried to understand the role of B cells and antibodies in the tumor microenvironment. The other project I've worked on involves bulk RNA sequencing and single cell sequencing on tissue samples from patients from the operable cohorts of the trial who have also received immunotherapy. We're trying to understand whether any immune cells correlate with a good outcome or a poor outcome for patients.

What fascinates you about that question?

I think it's the promise of treatment, especially for esophageal cancer. So many cancers that are being treated with checkpoint inhibitors have had dismal prognoses in the past. There's great interest in trying to help more patients benefit from this treatment because, at the moment, the response rates are still quite low. From a scientific perspective, I find it absolutely fascinating that, using computational techniques, you can look into a cancer. It's just really exciting to me.

Do you feel like your clinical background brings something a little different?

I think appreciating how fortunate we are to be able to work on these trial samples. Behind every sample is a whole patient, is a whole person. I'm not sure that's always helpful to remember because that makes the work maybe even more stressful than it already is, but I think it's also important to remember what it is we're working on.

> "Science is missing out on many great talents who just don't have the opportunities to pursue higher education. It's a loss for everyone."

Where is that research heading?

Checkpoint inhibitors have only been approved in the context of esophageal cancer since 2021. For many cancers that is still a very new thing and not as much part of clinical practice as many other treatments. I think the exciting new development is being able to better understand what underlies the response to the treatment and being able to combine it with other treatments. There's loads of interesting work coming out of combining that with other approaches across cancers and understanding the basic science behind how these treatments synergize so we can use them in a sensible way.

What country would you most like to visit?

My hometown has quite a large Japanese population. I got to learn Japanese in school, and we have quite a lot of local Japanese culture in my hometown. I think it would be fantastic to visit Japan.

Is there something you think we could do better in the science world?

I don't really blame this on science, but I feel like a big issue is the question of access. I've been very lucky in my life. I've been given great opportunities. But there are many people who never get these opportunities. Widening access to higher education, especially on a graduate level, is something I think should be a big focus. Science is missing out on many great talents who just don't have the opportunities to pursue higher education. It's a loss for everyone.

Jenna AbuSalim

MD-PhD candidate
Ludwig Princeton
Joshua Rabinowitz laboratory

Can you tell us a bit about yourself and your family?

I am from a small town in southern Illinois called Effingham—born and raised there. I have a younger brother who is a nuclear engineer in Knoxville and an older sister who lives in Louisville and is a high school special ed teacher. Mom's a recently retired pediatrician and my dad is a real estate developer for rural areas. And I'm married, I should say. That's my family now.

When did you get married?

I got married in 2020, we had a lovely pandemic wedding. It was 19 people in a small Italian restaurant, and we had as much food as you could possibly imagine, which was phenomenal.

When did you become interested in science?

My mom was a pediatrician, so that was my initial inspiration, but I actually went to college as a music major. Indiana University has a great program, it's called a BSOF, which is a Bachelor of Science in Music and an Outside Field, so I did Chemistry and Oboe Performance. I had a fantastic organic chemistry teacher who took me under his wing, and I joined his lab. That's when I started to fall in love with the research process.

What are you focused on in your research these days?

I focus on how diet affects the gut microbiome, on how it affects the compounds and metabolites the bacteria produce. Are there differences between different types of proteins, dietary processing, as well as different amounts of fiber in your diet? And how does it impact the overall health of your gut microbiome?

What will your field of research have achieved in the next 20 to 30 years?

My hope is that we will give doctors specific therapeutic dietary knowledge. There are some things, like having fiber in your diet, that we've known for years. But what types of fiber? Which proteins? How much fat, how much protein—those kinds of things. I think we'll be able to say "Okay, if you're going to go on this cancer immunotherapy, you might want to have these nutrients in your diet." It'll be another way to tackle diseases, in addition to all the pharmacotherapies we have now. That's what excites me.

What's the best career advice that you've ever received?

I volunteered for hospice for a couple of years when I was in college and met this amazing man. He had ALS. He supposedly had just six months, but I actually got to stay with him for two years, and it was an amazing relationship. He probably had four different careers in his life and was very inspiring. He said to me, "Jump at any opportunity. If it's outside of your field of interest or comfort zone, the first couple of months might be really painful, but if you're dedicated and willing to start off in a place with nothing, you will gain so much." And I really started. I was in music, sort of thinking about medicine, and I jumped at the opportunity to join an organic chemistry lab, and then I knew I wanted to do more biologically related things, so I jumped at a cancer radiation lab and learned so many things. And now I'm in whole body metabolism.

What do you think could be improved in our approach to scientific research? Is there anything we could do better?

I'm in an ethics class now and something that we discussed in that class that I think would be excellent is if we had a negative results journal. There are so many labs that have these great stories, but they're just negative stories. There's nowhere really to publish that work. So other people spend time and resources on the same hypotheses because they don't know that somebody else has already proven them untrue.

"It would be excellent if we had a negative results journal. There are so many labs that have these great stories, but they're just negative stories. There's nowhere really to publish that work. So other people spend time and resources on the same hypotheses because they don't know that somebody else has already proven them untrue."

Do you still play music?

Absolutely. The New Jersey Intergenerational Orchestra here in New Jersey. I think the youngest member is around five and we've got a couple people in their 80s. There are people who've been playing for a year or two, then 10 years, and then people like me who have been playing for most of our lives. We have a concert in April.

Ángel Álvarez-Prado

Postdoctoral fellow

Ludwig Lausanne
Johanna Joyce laboratory

Tell us a bit about where you were born and raised.

I was born in Salamanca, a small town in the center west of Spain. It's a very old city, dating to pre-Roman times—I think it's from the seventh or eighth century BC. It has very characteristic architecture. Everything is dominated by a Baroque style and built of a special type of stone, called "Piedra de Villamayor," which gives the buildings a golden tone, so they shine beautifully under the sunlight. It's really a lovely place: small, very easy to live in, and culturally very, very rich. It hosts one of the oldest Universities in Europe. But I was raised in Guadalajara, a green, quiet and small town close to Madrid.

How did you wind up in science?

My interest in science began a very long time ago, probably high school. As a teenager, I was a bit geeky, always attracted by anything related to science and technology. I remember very well, for example, when the first draft of the human genome was published. I was 12 or 13 years old and it coincided with the first time I watched the movie Gattaca. I remember being thrilled by the possibility of manipulating the code of life, but at that point it looked like science fiction. Then, suddenly, the news was "Look, we now have read the human genome!" It was completely mind-blowing, I needed to know more about it.

What did you study for your PhD and what brought you to Lausanne?

I was always very interested in the biology of small things. I was fascinated by how we can operate as large organisms, but based on tiny molecules that work in a very coordinated fashion. An internship I did in my last year in college really shaped my taste for immunology and cancer. I was working on lymphoproliferative disorders, and I decided that I wanted to work in B cells specifically—and at the intersection of cancer biology and immunology. So I applied to the "B lymphocyte biology" lab, led by Almudena Ramiro at the Spanish Center for Cardiovascular Research (CNIC) in Madrid, where I developed my MSc and PhD theses, both successfully defended at Universidad Autónoma de Madrid. My PhD focused on understanding the molecular underpinnings of a fundamental process—antibody diversification—and how it can be derailed and lead to the development of

lymphomas. Back then, when I thought about cancer, I would only think about the malignant cells. But Karin de Visser gave a talk at CNIC that really opened my mind, introducing the concept of the "tumor microenvironment" and how immune cells can not only attack the tumor but also be hijacked by cancer cells to foster tumor progression, therapy resistance and metastatic spread. I was missing the big picture, the idea of tumors as whole ecosystems. I decided almost immediately that for my postdoctoral research, I wanted to work on the tumor microenvironment and specifically on its immune component. And when I looked into the literature for labs doing frontier research in this field, Johanna Joyce's was the obvious choice. I was extremely fortunate to be selected for her lab.

What has the focus of your postdoctoral research been in Johanna's lab?

In very general terms, I study the immune microenvironment of brain tumors, both primary tumors—glioblastomas—and brain metastases, with the idea of manipulating it for therapy. I've been working mostly on two different projects. The first one tried to answer a very simple question: How does the genetic makeup of cancer cells shape the composition and phenotype of immune cells in the microenvironment of brain metastatic tumors? Understanding this would have obvious implications for developing personalized immunotherapies, right? This study was published last year, and there are several spin-off projects derived from this research that I'm now working on.

The second project focused on glioblastomas. These are deadly primary brain cancers with a dismal median survival of around 15 months. They are very, very difficult to treat, firstly, because of their location, and secondly, because they're extremely heterogeneous. Within the same tumor, you can find cells with different genetic and epigenetic alterations belonging to different transcriptional subtypes, which makes it very difficult to find a single therapy able to eradicate all malignant cells. In addition, glioblastoma tumors present a very complex immunosuppressive microenvironment, which supports tumor growth and contributes to therapeutic resistance. This second project aims to reprogram the way in which cancer cells communicate with the immune microenvironment. Cancer cells are tricking the immune system. They're saying, "I'm the good guy. Don't attack me, don't kill me, help me grow." What I'm trying to do, by exploiting an innate immunity checkpoint, is to alert the immune system and foster the recruitment of effector immune cells and to also rewire the cells that are already in place—mostly tumor-associated macrophages—to trigger an antitumoral response.

Where do you see your research heading now, as you complete your postdoc?

I think my research will head towards developing personalized immunotherapeutic approaches for brain tumors informed by their genetic and microenvironmental landscapes. I'm now generating a series of mouse models that we plan to use as preclinical platforms to explore if we see differences in responses to immunotherapy depending on the mutational profile of the tumors plus the associated composition and phenotype of their immune microenvironments. This will be one of the pillars of my future lab, but I have many other ideas that I'm also eager to explore.

Where do you see tumor immunology evolving over the next 20 or 30 years, and what do you think it will have achieved by then?

That's tough to answer. I think that spatial 'omics and artificial intelligence are going to play a major role shaping the way in which we study cancer biology and develop new

therapies. And I think there will be significant advances in data-driven personalized medicine approaches. Artificial intelligence will allow us to exploit the huge amount of clinical, molecular and spatial data we're collecting to develop more effective customized immunotherapies, which will prove more and more effective as we advance our understanding on the mechanisms of immunosuppression that are at play in different cancers. Overall, I'm optimistic that we will have more efficacious treatments for cancer patients and I'm convinced that the immune system will stay at the core of these novel therapeutic approaches.

What are your avocational interests?

I love biking. I bike a lot, road biking mostly. Here in Switzerland, it's truly amazing. The landscapes that we have here are stunning. It's a bit demanding because it's quite hilly, but I think it's good exercise and also a fantastic way to clear your mind and relax. Sometimes new ideas come up during my cycling because it's a time in which I'm free of any other demands or time constraints. The same holds true for hiking, which I greatly enjoy as well.

I also love photography, particularly street photography. I frequently go to exhibitions and I have a ton of photography books at home. I shoot quite a bit, so you will frequently see me in the streets with my camera trying to capture interesting moments. And it has a similar effect to biking and hiking—it's like meditation.

What is the best career advice you've ever received?

I've been very lucky to have excellent mentors at all stages of my training, but I think my favorite bit of advice is something my PhD director told me once, that the quality of a scientist is usually directly proportional to their ability to say "No." We would all love to say yes to every request—to review a paper, to go to a conference, maybe to start a new exciting collaboration—but the reality is that time is finite. We have a limited number of hours per day and so many things on our plate that it's very important to prioritize and, whatever we do, do our best at it.

What would you change about the way we conduct science, if you could?

To begin with, the publication system, which is totally broken. We pay for publishing, we pay for accessing most of the journals, but we review for free. We give impact factors an excessive importance when we evaluate scientists, this is something that we should change. I think peer review must stay and scientists must be evaluated based on their work but it is very detrimental that we concede this dominant position to a few editorial companies and let them have such a large impact on our careers.

Come back to the way in which we used to do science in the past. When I read old papers, I'm always fascinated by how elegant and how simple the experiments were. My feeling is that now we are industrializing science. Now every paper needs to have the latest and fanciest technologies. It needs to have 25 figures. It needs to be extremely complex. I think this is because we are just spinning the wheel faster and faster, so we need to produce more, to produce fancier, to finish faster. So as we have more and more technology, I feel that we are devoting less and less time to just sitting down and thinking. If you see a Nature paper from the '50s, '60s, it's usually three figures and it's the three key experiments. And the experiments are beautiful, and the results are clear, and that was enough. Scientists in the past had the privilege of having a lot more time to just think, to be bolder in the ways in which they would design an experiment. I would like to come back a bit to this more romantic view of being a scientist.

How do you currently use artificial intelligence in your work, if you do at all, and what other potential applications do you envision for AI in the future of cancer research, prevention, diagnosis or treatment?

DAVID GFELLER
Ludwig Lausanne

Cancer research is undergoing a transformative shift towards a data-driven science. The pivotal role of AI in this evolution is undeniable. In our research, we prioritize creating computational models that utilize AI's predictive capabilities, while also ensuring they remain interpretable. This emphasis on interpretability aids in comprehending the rationale behind AI predictions.

MARKUS MÜLLER
Ludwig Lausanne

We showed that machine learning can improve the prioritization of HLA-I neoantigens as targets for cancer immunotherapy. These predictors are included in our NeoDisc neoantigen prediction pipeline and used in personalized cancer vaccine and T cell programs at the CHUV. Future patient data will allow us to improve and extend the predictors to HLA-II binding neoantigens.

PETER SORGER
Ludwig Harvard

We use AI and machine learning (AI/ML) to study the tumor-immune microenvironment in highly multiplexed and traditional tissue images. AI/ML can be highly effective in this setting, revealing morphological and molecular features that are not always discernable to human experts. We are aiming for machine-human collaboration that uses AI to accelerate scientific discovery and improve diagnosis.

NEHA WALI
Ludwig Oxford

I don't currently use AI in my bioinformatics projects. However, there are myriad applications for it in research and medicine. For example, AI can help clinicians diagnose cancers by analyzing imaging scans. It can also assist researchers to identify biomarkers of therapeutic response in large clinical trial datasets.

Ask a scientist

We are using AI to read the regulatory code of the genome - how transcription factors, the "master regulators" of cell type identity, activate regulatory elements throughout the genome, and how such regulatory elements modulate expression of their target genes.

JOANNA WYSOCKA
Ludwig Stanford

CHRISTOPHER DOUVILLE
Ludwig Johns Hopkins

We find machine learning and large data sets being applied to a wide variety of problems in our group but we find it is critical to remember that these powerful tools are not magic and NOT a substitute for a sound hypothesis, a deep understanding of the data and a recognition of the limitations of the experimental data.

My lab is developing and applying chemical AI to mass spectrometry-based metabolomics data to discover new human and microbiome-derived metabolites. We are excited both about the possibility of using AI to extract more value from existing or routinely-collected datasets, and allowing us to collect even larger datasets through more complex and ambitious experiments with the expectation that we will be able to sift through huge amounts of data to ultimately arrive at new biological and clinical insights.

MICHAEL SKINNIDER
Ludwig Princeton

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