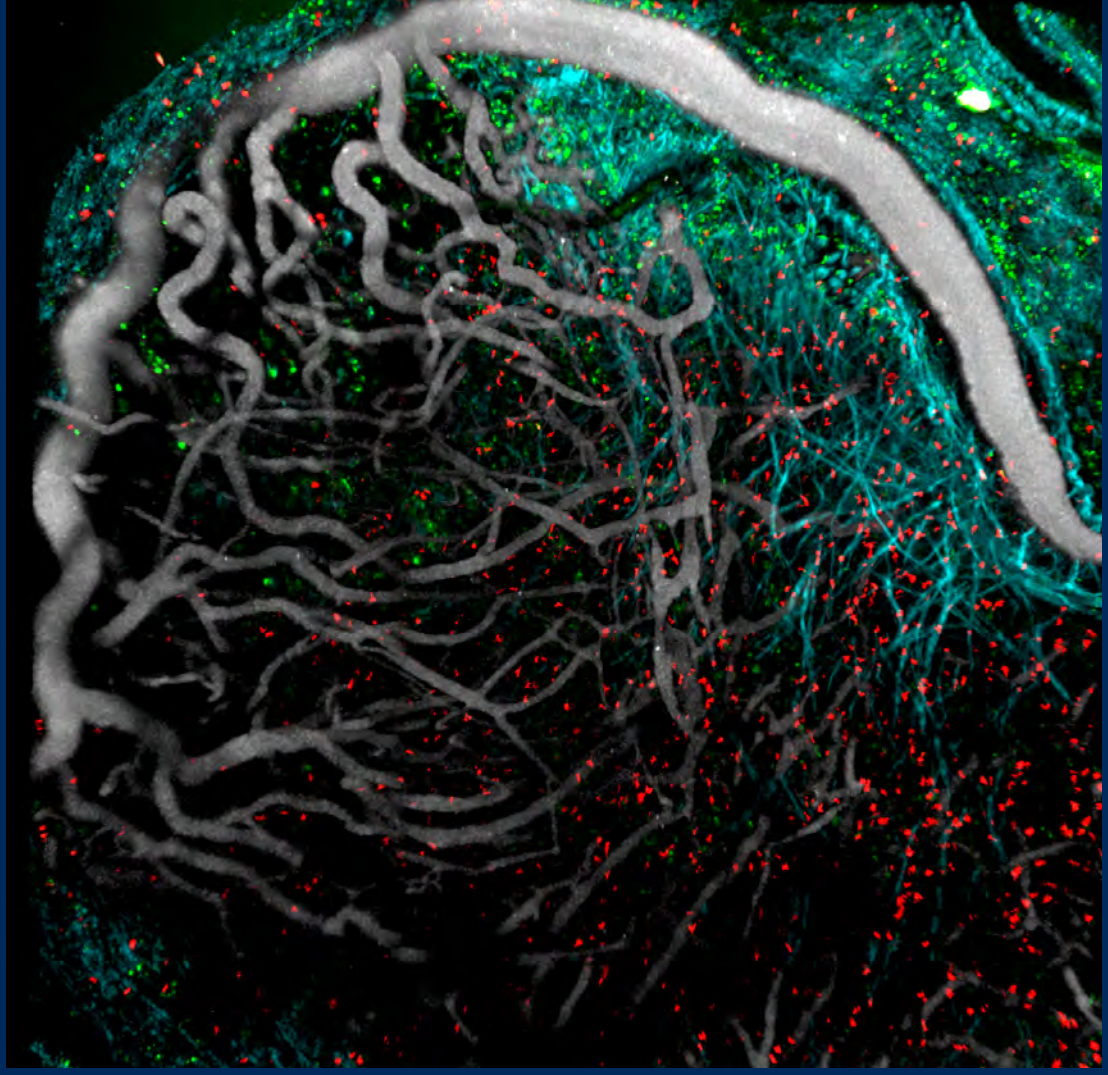


May 2025

Ludwig Link

LUDWIG CANCER RESEARCH

LIFE-CHANGING SCIENCE

Welcome

Unmesh Kher
Editorial Director

Ludwig Link veterans might have noticed that bright micrographs have lately turned up—with rising frequency, in issue after issue—in the pages of this magazine.

Yet the Communications team's appetite for pretty pictures was not sated by these servings alone. So we came up with a new feature for the Link and asked researchers across the Ludwig community to share with us striking images captured whenever in their labs and tell us a bit about them.

And share they did. Flip to the back of this book (page 24) to check out what your colleagues served up for the debut of the Link's photo gallery. Alternatively, you could work up an appetite for that feast by first perusing our research news section. Aside from its own striking pictures, the section has briefs on everything from the clinical validation of liquid biopsies to better manage stage II colon cancer treatment to the remarkable results of an early trial examining a CAR-T therapy for an intractable pediatric brain cancer.

Other news in this issue covers the Ludwig Institute's participation in the Weill Cancer Hub East, a $125 million research program at the interface of diet, metabolism and cancer immunotherapy. We also report on a Ludwig Oxford program exploring why and how a subset of generally indolent myeloproliferative neoplasms evolve into aggressive leukemias (page 8). Aside from that, we introduce you to a new director of Ludwig Chicago (page 7) and ask you to join us in congratulating four Ludwig scientists honored for their outsized contributions to cancer research (pages 5-6).

Welcome to the May issue of the Ludwig Link!

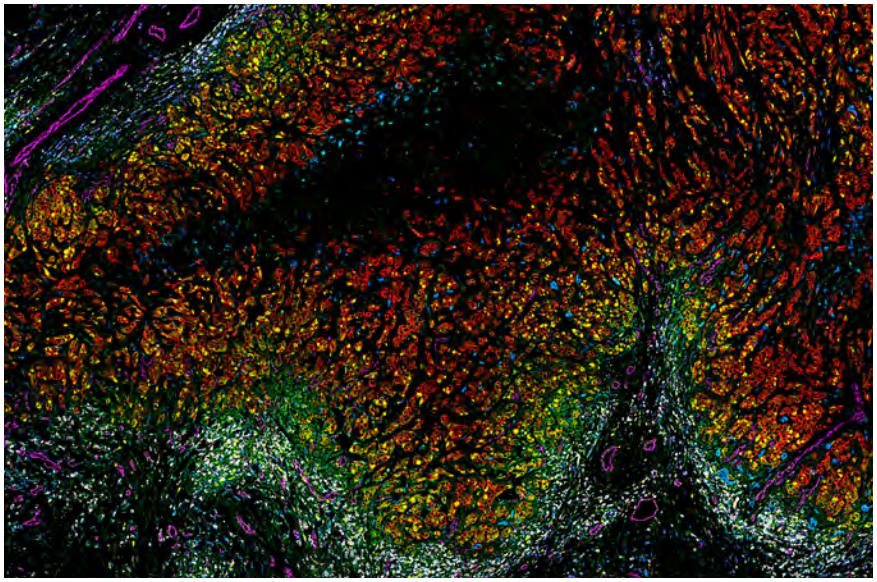
Unmesh Kher
Editorial Director

On the cover

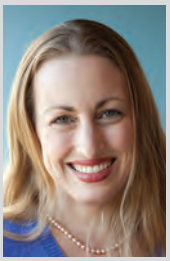
This is an intravital 2-photon image of a mouse melanoma ten days post-engraftment, with CD8+ tumor-infiltrating lymphocytes (TILs, orange and red), dendritic cells (green), blood vessels (Qdot, gray), and collagen fibers (second harmonic generation, cyan). This image was kindly submitted to us by Ludwig Lausanne's Ping-Chih Ho and Stefania Vilbois for a new feature of striking images generated by Ludwig laboratories—one we hope to run routinely in future issues of the Ludwig Link. Learn more about what you're seeing here on **page 29.**

Image by Stefania Vilbois.

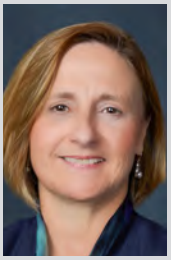
In this issue



FEATURED RESEARCH



Michelle Monje



Crystal Mackall

Researchers led by Ludwig Stanford's Michelle Monje and Crystal Mackall reported encouraging final results for one arm of an ongoing Phase 1 trial evaluating the use of CAR-T cells directed against the ganglioside GD2 for H3K27M-mutated diffuse midline gliomas, a family of aggressive pediatric brain cancers. **PAGE 23**

Douglas Hanahan recognized for extraordinary achievement in cancer research

Ludwig Lausanne's Douglas Hanahan received the 2025 Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research. This prestigious award recognizes scientists of international renown who've made major contributions to basic or translational cancer research. Doug certainly fits that bill. As a young scientist at Cold Spring Harbor Laboratory in New York in the 1980s, Doug created one of the first mouse models of cancer, reporting his landmark accomplishment in a single-author publication in *Nature*. Working with the late Judah Folkman, he went on to identify an "angiogenic switch" that triggers the blood vessel growth essential to tumorigenesis and explored the disruption of angiogenesis for cancer therapy. He has since used his models to investigate the stages of cancer progression, the tumor microenvironment, drug resistance and tumor immunology. Doug is also noted for authoring with Ludwig MIT Co-director Robert Weinberg *The Hallmarks of Cancer*, a landmark perspective on cancer biology published in *Cell* in 2000. Updated in 2011 and 2022, the essays established an unprecedented conceptual framework for understanding the cellular and molecular underpinnings of cancer. The papers remain among the most influential publications in modern cancer biology. Doug was honored and gave an award lecture during the AACR Annual Meeting in Chicago in April and was slated to be similarly honored at a special award ceremony in May in Trento, Italy.

Douglas Hanahan

Bradley Bernstein elected Fellow of the AACR

Ludwig Harvard's Bradley Bernstein was elected Fellow of the Academy of the American Association for Cancer Research. Each year, the AACR nominates a handful of researchers and vets them through a rigorous, peer-reviewed process that ensures only those who have made profound and lasting contributions to cancer research and related fields are ultimately elected to its Academy. Brad and his colleagues pioneered the mapping and functional analysis of the epigenome, the full spectrum of chemical modifications made to DNA and its histone protein packaging—collectively known as chromatin—that regulate the expression of the genome. His studies over the past couple of decades have illuminated on a large scale how epigenetic modifications inform chromatin structure, maintain the pluripotent potential of stem cells, orchestrate embryonic development and, when disordered, alter gene expression to drive cancer. One of 33 researchers elected to the AACR Academy's class of 2025, Brad was recognized by the Academy for his "seminal contributions to cancer epigenetics, including the discovery of bivalent chromatin domains that regulate developmental gene activation and the role of IDH mutations in disrupting chromosomal topology, resulting in the establishment of new mechanisms by which to characterize tumors and optimize therapeutic strategies."

Bradley Bernstein

Two Ludwig scientists recognized for lifetime achievement

Rakesh Jain

Rakesh Jain honored by the AACR

Ludwig Harvard's Rakesh Jain was honored in April with the 2025 AACR Award for Lifetime Achievement in Cancer Research in recognition of scientific contributions that have advanced our understanding of the tumor microenvironment and its role in cancer progression and responses to therapy. In issuing its award, the AACR made special mention of his formulation and validation of the vascular normalization hypothesis, which it noted "reshaped the use of antiangiogenic therapy and led to FDA-approved drug combinations" for cancer therapy. Rakesh's research established that abnormalities in the blood and lymphatic vessels in tumors fuel cancer growth and metastasis, limit the delivery of drugs into tumors and diminish their efficacy. He subsequently proposed that antiangiogenic agents—originally developed to block blood vessel formation—could "normalize" blood vessels and showed in preclinical studies not only that this was true but that such therapies improve the delivery and efficacy of multiple drugs as well as the infiltration of anti-cancer immune cells into tumors and their stimulation by immunotherapy. Several clinical trials, some led by Rakesh in partnership with clinical researchers, have since led to the regulatory approval of various drug combinations with antiangiogenic therapies for the treatment of many cancers. Rakesh, who gave an award lecture at the AACR's Annual Meeting in April, is the third member of the Ludwig community—along with Ludwig Lausanne's Douglas Hanahan and Ludwig MIT Co-director Robert Weinberg—to have received this prestigious award.

Colin Goding

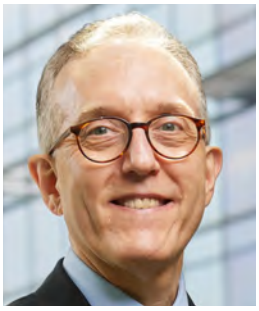
Colin Goding honored by the Society for Melanoma Research

Ludwig Oxford's Colin Goding was honored with a Lifetime Achievement Award from the Society for Melanoma Research, an organization of clinicians and scientists dedicated to alleviating the suffering caused by melanoma and building bridges between basic and clinical researchers. Colin's laboratory was the first to propose that the microenvironment, rather than genetic mutations, is the primary driver of melanoma invasiveness and progression. He and his colleagues have shown, most notably, that the microphthalmia-associated transcription factor (MITF) plays a central role in altering melanoma cell phenotypes. MITF-low cells are drug-resistant, slow-cycling, tumor-initiating and invasive, while cells that express MITF at high levels tend to have a proliferative phenotype. Colin's studies have examined how the expression and activity of MITF are regulated and how the protein integrates signals from the local environment to modulate melanoma cell phenotypes. This work has enriched our understanding of how microenvironmental stressors that switch cells from a proliferative to an invasive state do so by reprogramming protein translation and metabolism to impose a starvation or 'pseudo-starvation' phenotype. These studies are also of value to developing new approaches to treating the aggressive skin cancer. More broadly, they open a window into understanding how and why stem cells are established in melanoma and how microenvironmental forces drive cancer progression in this and other cancers.

Four research institutions launch the Weill Cancer Hub East

In March, the Ludwig Institute for Cancer Research joined Princeton University, The Rockefeller University and Weill Cornell Medicine to launch the Weill Cancer Hub East. Seeded with a $50 million gift from the Weill Family Foundation and supplemented with $75 million from the partner institutions, the Hub will over the next decade deploy interdisciplinary teams at the interface of tumor immunology and metabolism to discover and develop new strategies for cancer therapy. Its researchers will explore how metabolism shapes the tumor microenvironment and how the food we eat and the microbes that aid its digestion influence cancer therapy. The Hub's researchers will also evaluate how new drugs, like GLP-1 agonists, might affect cancer progression and treatment and examine strategies to modulate metabolism and microbiomes to reprogram the tumor microenvironment to improve the efficacy of cancer therapy. It will also conduct clinical trials to test its clinical hypotheses in patients. The Hub's research program dovetails neatly with that of the Ludwig Institute, and not only due to the Institute's continuing leadership in tumor immunology and immunotherapy. The Ludwig Princeton Branch, which is affiliated with RWJBarnabas Health and Rutgers Cancer Institute, is a global hotspot of research on cancer metabolism and metabolomics. It is already participating in a research initiative the Institute launched last year across Ludwig Branches, Centers and host institutions exploring the links between diet and immunometabolism. Further, two Ludwig Institute researchers—Ludwig Princeton Director Joshua Rabinowitz and Co-director of the Ludwig Collaborative Laboratory at Weill Cornell and Board Member Jedd Wolchok—are on the scientific steering committee that will oversee the Hub's research program. Looks like we'll be playing a prominent role in this exciting new endeavor.

Joshua Rabinowitz



Jedd Wolchok

People on the move

Jeffrey Rathmell appointed co-director of Ludwig Chicago

Jeffrey Rathmell joins our community this summer as a director of the Ludwig Center at the University of Chicago, where he will also chair the Ben May Department for Cancer Research. He joins us from Vanderbilt University, where he currently holds the Cornelius Vanderbilt Chair in Immunobiology at the School of Medicine. Jeff's expertise is in immunometabolism, which explores the molecular crosstalk between systemic and tissue-specific metabolism and immune cells. His laboratory has made notable contributions to our understanding of the dynamism and functional significance of T cell metabolic programs, uncovering clues to their potential manipulation for the treatment of cancer and inflammatory diseases. It has also made major discoveries about how metabolic states such as obesity influence the anti-tumor activity of T cells and macrophages. Jeff completed his doctoral studies in immunology at Stanford University, his postdoctoral stints in immunology and cancer biology at the University of Chicago and University of Pennsylvania and joined the faculty at Duke University before moving to Vanderbilt in 2015. He will take the helm at the Chicago Center on July 1, serving as co-director with Ralph Weichselbaum.

Jeffrey Rathmell

A concerted assault on myeloproliferative neoplasms

Factors contributing to MPN progression

- Mutation of epigenetic regulators
- Dysregulation of p53, DNA damage response and genetic instability
- Aberrant TME: inflammation, fibrosis and immunity
- JAK/STAT signaling pathway activation
- Perturbed haematopoietic differentiation

The Ludwig Oxford Branch launched this year a research program focused on myeloproliferative neoplasms (MPNs), a family of typically slow-growing blood cancers that in some patients transform into aggressive leukemias. Known as blast-phase MPN (BP-MPN), this transformation of the disease has an associated life expectancy of 6-12 months. All three types of MPN—essential thrombocythemia (ET), polycythemia vera (PV) and low-risk myelofibrosis—are in most cases indolent cancers, accompanied by some symptoms but only a modest reduction in life expectancy. About one in three MPN patients, however, develops a high-risk type of myelofibrosis with a median survival of 5-7 years. About 20% of the latter, and 1-5% of ET and PV patients, progress to BP-MPN, for which there is currently no treatment that improves outcomes.

MPNs are driven by mutations in hematopoietic (blood-forming) stem cells to three genes whose products participate in signaling through the thrombopoietin receptor (TPO-R) via the JAK-STAT pathway: JAK2 (the famous *JAK2*V617F mutation), calreticulin *(CALR)* and TPO-R *(MPL)*. Though drugs that inhibit JAK2 control MPN symptoms in most patients, they do not stall the disease or prevent progression to BP-MPN and have little efficacy against the secondary leukemia. That escalation is fueled by mutations to the tumor suppressor *TP53*, epigenetic regulators (like *EZH2, ASXL1*)—which chemically tag DNA and histones to regulate gene expression—and transcription factors expressed by myeloid cells (such as RUNX1). Gene copy number alterations also drive that transformation and could offer clues to the development of therapies for BP-MPN.

The development of such therapies is a critical unmet need in clinical oncology. So is a better understanding of risk factors for the progression of MPNs to BP-MPN, about which precious little is currently known. Identification of those risk factors would not only improve monitoring of patients at high risk for secondary leukemia, but might enable the development of new therapies for BP-MPN and interventions to capture such cases early and perhaps even prevent disease progression.

The MPN Program at Ludwig Oxford, which will be conducted in collaboration with researchers at the MRC Weatherall Institute of Molecular Medicine (MRC WIMM) and the Radcliffe Department of Medicine at the University of Oxford, addresses these needs. It aims to identify mechanisms and related biomarkers of disease progression and to use such insights to develop therapeutic strategies to treat and prevent BP-MPN. This requires a defined spectrum of expertise and the development of research tools, disease models and patient cohorts—all of which are available or can be developed at the Oxford Branch and MRC WIMM. The initiative especially benefits from a critical mass of scientific talent in epigenetics, cancer plasticity, signaling and p53 tumor suppressor mechanisms already assembled at the Oxford Branch. Further, its translational objectives are aided by the presence in its leadership of clinicians experienced in the care of MPN patients.

Overseen by four program leads—physician-researchers Ludwig Oxford's Bethan Psaila, Stefan Constantinescu and MRC WIMM's Adam Mead along with Ludwig Oxford Director

Program leads

Bethan Psaila
Tumor microenvironment in MPNs, fibrosis, organoid models

Stefan Constantinescu
JAK/STAT signaling, epigenetics, MPN and leukaemias

Adam Mead
Normal and malignant stem cell biology, p53 and MPN progression

Xin Lu
p53, genetic instability

Investigators

Benjamin Schuster-Böckler
Computational, cancer (epi) genetics, mutagenesis

Skirmantas Kriaucionis
Cancer epigenetics

Chunxiao Song
Chemical epigenetics

Jens Rittscher
Biomedical imaging multi-modal data analysis applied to cancer

Daniel Royston
AI/machine learning, translational histopathology applied to MPN

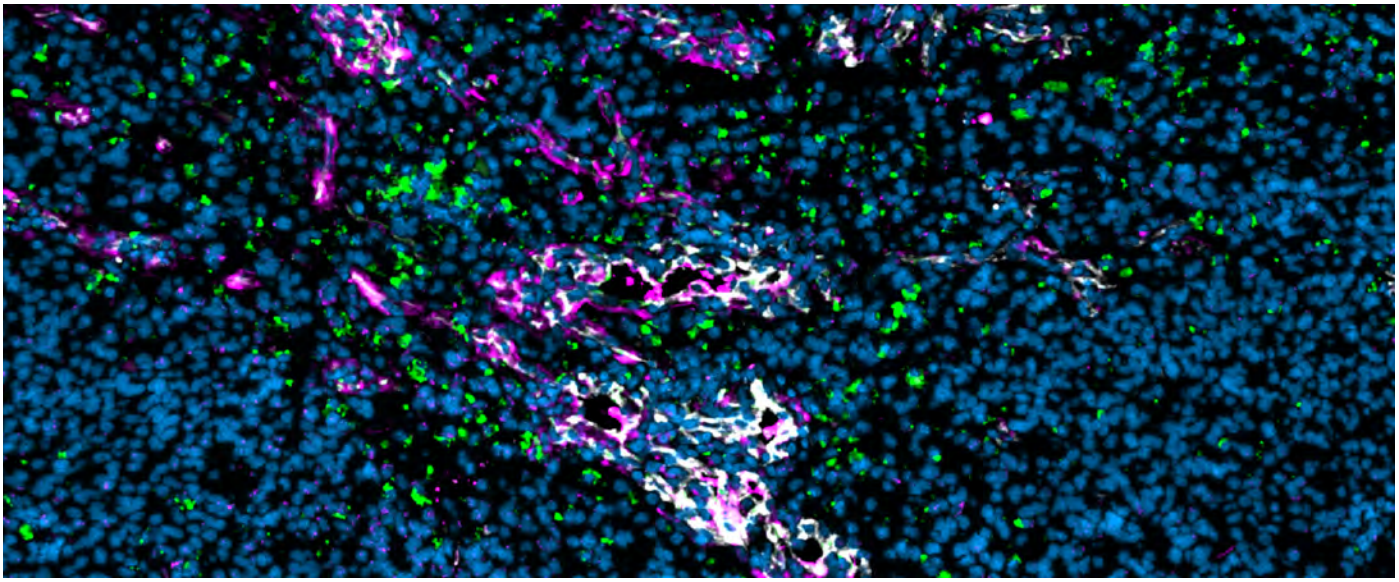
Yang Shi
Epigenetics, differentiation therapy

Marketa Tomkova
Computational, mutagenesis, epigenomics

Benoît Van den Eynde
Tumor immunology and micro-enviroment

Xin Lu—the MPN initiative has three arms that pull together the required scientific expertise (see graphic). One is dedicated to exploring the epigenetics, molecular biology and aberrant signaling underlying MPN progression to acute leukemia and testing the veracity of its mechanistic models, which will inform translational efforts. A second arm is dedicated to the discovery of biomarkers and the development and validation of translational tools and technologies—ranging from imaging to machine learning to liquid biopsies and epigenetic mapping—for their efficient detection. The third focuses on obtaining samples from large cohorts of patients with myelofibrosis and the development of human iPSC-based xenograft and human organoid models to vet the clinical hypotheses generated by the project.

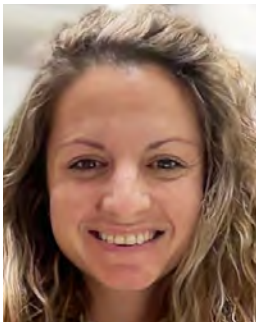
The Link thinks science just got even more exciting at the Oxford Branch.



Leire Bejarano

A tissue section from a glioblastoma patient, showing all cells (blue; nuclei stained with DAPI), endothelial cells (white; CD31), myeloid cells (green; CD68); and fibrinogen (pink), which is a marker for vessel leakiness.

Johanna Joyce



Leire Bejarano

A vascular atlas of primary brain tumors and brain metastases

Primary and metastatic brain tumors show considerable variation in their immune microenvironments, and these differences are of significant relevance to the success of novel therapeutic strategies now under development. The tumor vasculature, a key component of that microenvironment, serves as an active gatekeeper of immune cell infiltration and a mediator of immunosuppression and drug resistance. Researchers led by Ludwig Lausanne's Leire Bejarano and Johanna Joyce reported in an *Immunity* paper published online in March (and as the cover article of the April issue) an in-depth, comparative analysis of individual cells—focusing on endothelial and mural cells—that compose the blood-brain barrier across healthy brain tissue, IDH-mutant low grade gliomas, high-grade glioblastomas (GBMs), and brain metastases arising from various cancers. Integrating immunofluorescence microscopy and spatial analyses with single-cell transcriptomics and bulk RNA sequencing, the researchers found that alterations in tumor-feeding blood vessels are far more pronounced in metastatic brain tumors than in gliomas, with the former showing higher permeability and interactions with distinct immune cell populations. Within primary brain tumors, GBMs show more pathological alterations in their vasculature than do low-grade gliomas. The high-resolution vascular atlas generated by this study lays the foundation for the development of novel vascular- and immune-targeting therapies for brain tumors.

Single-cell atlas of endothelial and mural cells across primary and metastatic brain tumor
Immunity, 2025 March 18

Tinkering with the machinery of antigen presentation

Patrolling CD8+ T cells recognize cancer cells by the snippets of cancer-specific proteins, or peptides, they present on HLA-I molecules on their surface. Cancer cells can escape such recognition when some component of the complex biochemical machinery behind this antigen presentation is compromised. These components include enzymes that cut proteins into short peptides, transporters that transfer those peptides from the cytoplasm into the endoplasmic reticulum, proteins that select the most suitable peptides for HLA binding and those that load them onto HLA molecules in preparation for their presentation. To study how deficiencies in distinct components of this machinery alter the spectrum of presented peptides on the cell surface, or "immunopeptidome", researchers led by Ludwig Lausanne's Michal Bassani-Sternberg and Ilja Shapiro used haploid cell lines, individually deleting eleven distinct genes encoding elements of this HLA-I peptide-presentation machinery. They reported in a March paper in *Molecular & Cellular Proteomics* the first collective qualitative and quantitative analysis of how the absence of individual components of that machinery affects the immunopeptidome. The absence of CALR (a folding protein), GANAB (a modifying protein) and ERAP1 (a transporter protein) induced significant, HLA allele-specific changes in peptide presentation levels. The findings should be of value to the creation of predictive tools to better prioritize the selection of antigens for personalized immunotherapies.

Deleterious knock-outs in the HLA class I antigen processing and presentation machinery induce distinct changes in the immunopeptidome | *Molecular & Cellular Proteomics*, 2025 March 18

Michal Bassani-Sternberg

Ilja Shapiro

Ping-Chih Ho

An immunometabolic hit on nasopharyngeal carcinoma

Preclinical studies have shown that acute asparagine deprivation can disrupt protein synthesis and intracellular signaling in a manner that compromises CD8+ T cell activation, but prolonged deprivation induces metabolic reprogramming that has the opposite effect. Researchers led by Ludwig Lausanne's Ping-Chih Ho and a colleague of his in Taiwan examined whether the latter effect might be harnessed to improve responses to immune checkpoint blockade (ICB) therapy. They reported in a March *Nature Metabolism* paper their findings from an open-label study that treated a few patients with recurrent metastatic nasopharyngeal carcinoma with L-asparaginase, which degrades asparagine and is used to treat blood cancers. Six patients resistant to anti-PD-1 ICB therapy received asparaginase injections for 3 or 5 days followed by the anti-PD-1 therapy pembrolizumab, and were compared with patients receiving only anti-PD-1 therapy. L-asparaginase fortified CD8+ T cell fitness and enhanced patient responses to ICB, with progression-free survival of at least 10.5 months compared to 2 months for ICB alone. Studies using mouse models of other cancers showed that asparaginase boosts the efficacy of anti-PD-1 by enhancing T cell activation. The researchers reported that acute and prolonged asparagine deprivation have opposite effects on mitochondrial health and biogenesis as well as T cell differentiation patterns and detailed the extensive metabolic rewiring that enhances T cell anti-tumor activity.

Asparagine deprivation enhances T cell antitumour response in patients via ROS-mediated metabolic and signal adaptations | *Nature Metabolism*, 2025 March 5


Yuxuan Wang


Bert Vogelstein


Jeanne Tie


Peter Gibbs

Monitoring circulating tumor DNA to improve stage II colon cancer therapy

About 15 years ago, researchers at Ludwig Johns Hopkins and their colleagues at the former Ludwig Melbourne Branch began a collaboration to determine whether circulating tumor (ct) DNA could consistently predict disease recurrence after surgery for stage II colon cancer—and do so well enough to safely spare patients unnecessary adjuvant chemotherapy (ACT). The researchers reported in 2022 in the *New England Journal of Medicine* that early data from the DYNAMIC trial, which enrolled 455 patients, suggested the answer is yes. ctDNA analysis accurately predicted risk of recurrence; and directing ACT only to ctDNA-positive patients reduced chemotherapy use overall without compromising recurrence-free survival. In March, the team—led by Ludwig Johns Hopkins' Yuxuan Wang and Bert Vogelstein with Ludwig Melbourne alumni Jeanne Tie and Peter Gibbs—reported in *Nature Medicine* their analyses of mature outcome data from DYNAMIC, including overall survival, ctDNA levels and ctDNA clearance in enrollees. Their analysis confirmed previous findings: after five years, both recurrence-free and overall survival were about the same for ctDNA-guided and standard management. The researchers found there's potential to further risk-stratify ctDNA-positive patients based on ctDNA burden and results after ACT ends. This research was partly supported by a five-year, $10 million program launched by the Conrad N. Hilton Foundation and the Ludwig Institute for Cancer Research for the prevention and early detection of colon cancer.

Circulating tumor DNA analysis guiding adjuvant therapy in stage II colon cancer: 5-year outcomes of the randomized DYNAMIC trial | *Nature Medicine*, 2025 March 7

Why brain metastases of breast cancer might resist a promising combo therapy

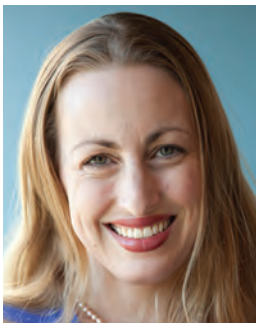
A study led by Ludwig Lausanne's Johanna Joyce and alumnus Vladimir Wischnewski explains why brain metastases of breast cancer are likely to resist combinations of stereotactic radiosurgery (SRS) and immune checkpoint blockade (ICB) therapy. Radiotherapy destroys tumors in part by stimulating T cell-mediated anti-tumor immunity, suggesting a potential synergy with ICB. Indeed, analogous approaches employing chemotherapy and ICB have improved outcomes for advanced triple-negative breast cancer. Johanna, Vladimir and colleagues developed a new protocol to deliver cranial SRS to mice to model the therapy in the clinic and examined the therapeutic effects of its combination with anti-PD-1 ICB therapy in a mouse model of breast-to-brain metastasis. They reported in a March issue of *Cell Reports* that intracranial metastases in these models cultivate a highly immunosuppressive microenvironment, undermining the desired therapeutic synergy of the combination. They showed that a subset of myeloid immune cells—neutrophils expressing the inflammatory mediators S100a8 and a9, and macrophages and their brain-resident versions, microglia, expressing Trem2—suppresses the anti-tumor activity of CD8+ T cells that infiltrate these tumors. This occurs even when the combination therapy destroys primary breast tumors. The study opens the door to developing targeted treatments to address a major unmet need of breast cancer care.

Johanna Joyce

The local microenvironment suppresses the synergy between irradiation and anti-PD1 therapy in breast-to-brain metastasis
Cell Reports, 2025 March 17

Inhibitory synapses excite the cells of diffuse midline gliomas, driving proliferation

Researchers led by Ludwig Stanford's Michelle Monje have previously shown that neural activity drives high grade gliomas (HGGs), aggressive, incurable cancers that include pediatric diffuse midline gliomas (DMGs) such as H3K27M-driven diffuse intrinsic pontine glioma, and glioblastomas (GBMs). Those studies revealed that excitatory glutamatergic synapses between neurons and glioma cells as well as paracrine signaling from neurons stimulate the proliferation of the cancer cells. Michelle and her colleagues explored how another class of neural synapses that form with glioma cells—GABAergic synapses—influence tumor growth. Employing electrophysiology, optogenetics and mouse models of HGGs, the researchers discovered that GABAergic synapses, though ordinarily hyperpolarizing and inhibitory, actually stimulate growth of DMG tumors—but not of GBM—in mouse models of the cancer. This, they reported in a February paper in *Nature*, is because DMGs, but not GBMs, express the NKCC1 chloride transporter. NKCC1 abnormally boosts chloride levels in DMG cells, making GABA depolarize the cancer cells. In line with this finding, the benzodiazepine drug lorazepam, which augments GABA signaling, accelerates DMG growth in mice and shortens their survival. The study points to new approaches to developing therapies for these cancers and suggests that DMG patients should probably avoid taking benzodiazepine drugs.



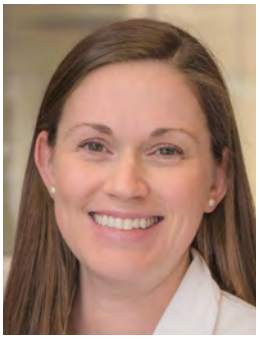
Michelle Monje

GABAergic neuron-to-glioma synapses in diffuse midline gliomas | *Nature*, 2025 February 19


Tyler Miller


Chadi El Farran

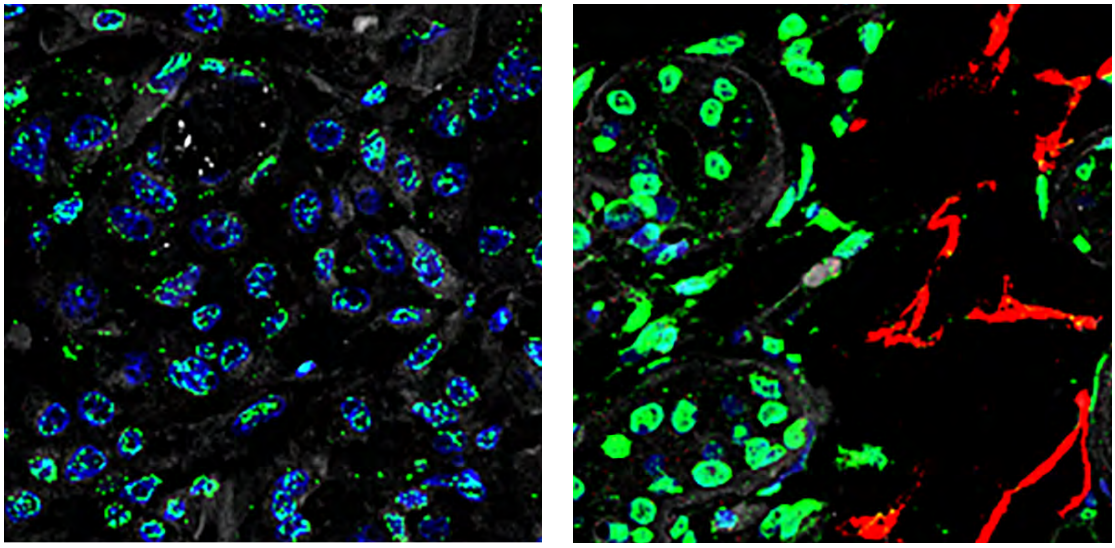

Bradley Bernstein


Jennifer Guerriero

A sweeping functional analysis of the myeloid landscape across human gliomas

Gliomas tend to be heavily infiltrated with myeloid cells. These immune cells play a pivotal role in tumor progression and the suppression of anti-tumor immune responses. To better understand the functional role of myeloid cells in gliomas, researchers led by Ludwig Harvard's Tyler Miller, Chadi El Farran and Bradley Bernstein—with important contributions from Jennifer Guerriero's lab at the Harvard Center—leveraged single-cell RNA sequencing data from 85 diverse gliomas and applied new computational methods to delineate four major immunomodulatory programs found across myeloid cell types. They then explored these programs using an integrated suite of methods, including lineage tracing, spatial transcriptomics, chromatin accessibility and experiments in *ex vivo* models. The researchers reported in a February paper in *Nature* their exhaustive characterization of the gene expression programs they had identified in myeloid cells. These include microglial inflammatory and scavenger immunosuppressive programs, which are both unique to primary brain tumors; and systemic inflammatory and complement immunosuppressive programs that are also expressed by non-brain tumors. The programs are driven not by the origins of myeloid cells or tumor mutational states but by microenvironmental cues—including tumor hypoxia, interleukin-1β, TGFβ and standard-of-care dexamethasone treatment—and can predict responses to immunotherapy and overall patient survival. The team's exhaustive characterization of mediating genomic elements, transcription factors and signaling pathways associated with each program uncovers potential strategies to manipulate glioma-associated myeloid cells to improve responses to immunotherapy.

Programs, origins and immunomodulatory functions of myeloid cells in glioma
Nature, 2025 February 26



Ana Chocarro-Calvo

A section through Clark level V primary human melanoma stained for DNA (blue), caveolin (CAV1; green) and perilipin (red), a protein associated with lipid droplets in cells. The images show low level CAV1 staining in the tumor core and greatly elevated CAV1 in cells in the vicinity of perilipin-positive adipocytes.

An interaction of nutrition and phenotype in melanoma metastasis

When expressed by cancer cells in specific phenotypic states, signaling through the AXL receptor—a tyrosine kinase—plays a role in cancer invasion and drug resistance in multiple cancers. Researchers co-led by Ludwig Oxford's Colin Goding, Ana Chocarro-Calvo and a colleague in Spain reported in a February paper in *Genes & Development* that, in a melanoma model, the AXL receptor is activated by exposure to human adipocytes and to oleic acid, a fatty acid abundant in both lymph and in adipocytes. AXL activation triggers the nuclear translocation of a β-catenin–CAV1 complex that is required for melanoma invasiveness. The researchers show that only undifferentiated melanoma cells expressing AXL at high levels engage in symbiosis with human adipocytes, in part by inducing lipolysis. This in turn leads to their uptake of fatty acids and the nuclear translocation of the β-catenin–CAV1 complex. They also showed that CAV1 is abundant in the nucleus of human melanoma cells near adipocytes. These findings underscore the importance of microenvironmental forces in cancer progression, illustrating how metastasis can be triggered by a specific nutritional stimulus delivered to cells in a receptive phenotypic state. Their findings, the researchers note, have implications for a broad range of other cancers that also express AXL and offer insights into the mechanisms underlying cancer cell dormancy and resistance to therapy.

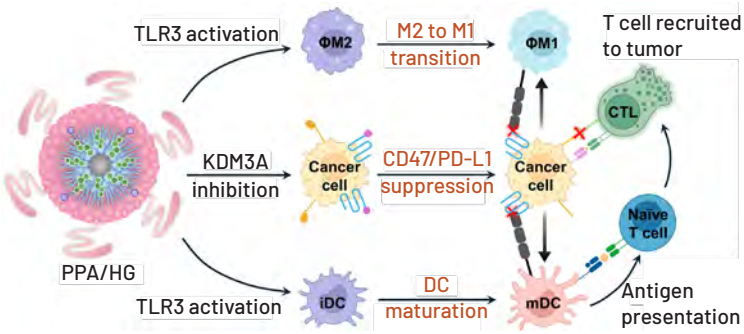
Colin Goding

Ana Chocarro-Calvo

Fatty acid uptake activates an AXL–CAV1–β-catenin axis to drive melanoma progression
Genes & Development, 2025 February 27

Wenbin Lin

Jing Liu



PPA/HG nanoparticle: Activation of dendritic cells and macrophages by the TLR3 agonist PPA and CD47 and PD-L1 downregulation by the KDM3A inhibitor HG synergistically induce antitumor immunity.

Two types of nanotech target tumors in distinct ways

Researchers led by Ludwig Chicago's Wenbin Lin reported in a couple of papers their design and preclinical evaluation of two types of nanotechnology with distinct mechanisms of action and potential as cancer therapies. A February paper in *ACS Nano* led by Wenbin and Jing Liu described a core-shell nanoparticle, PPA/HG, for immunotherapy. Wenbin, Jing and colleagues showed in their study that PPA/HG—comprising polyinosinic: polycytidylic acid (PPA) in the core and a cholesterol-conjugated prodrug of 3-(hydroxyolinoyl)glycine (HG) on the shell—persists stably in the blood and accumulates in tumors, where it releases its active ingredients. HG suppresses the expression by cancer cells of CD47—which would otherwise transmit a "don't eat me" signal to macrophages—as well as PD-L1, which inhibits CD8+ T cell targeting. PPA, meanwhile, activates toll-like receptor 3, which flips tumor-associated macrophages from a pro-tumor state to an anti-tumor state and drives the maturation of dendritic cells, which boost CD8+ T cell responses to tumors. Together, the two drive CD8+ T cell infiltration and deplete regulatory T cells, which suppress immune responses, in tumors. Systemic administration of PPA/HG inhibited tumor progression in mouse models of triple-negative breast cancer and pancreatic ductal adenocarcinoma with minimal side effects.

The other study, published in January, reported in *Advanced Science* the design and assessment of Hafnium (Hf)-based nanoscale metal-organic layers (MOLs) for cancer therapy. Hf-based MOLs enhance the therapeutic effects of tissue-penetrating X-rays by significantly amplifying the generation of hydroxyl radical and singlet oxygen species, which damage DNA and induce cell death. But their therapeutic efficacy is often compromised by low oxygen levels in tumors and the short half-lives of reactive oxygen species. In this paper, Wenbin and his colleagues detailed their design, production and evaluation of a MOL that generates superoxide anion (O_2^-) and releases nitrogen oxide (NO) in response to low-dose radiation. Sustained release of NO not only relieves intratumoral hypoxia to reduce resistance to radiotherapy but also reacts with O_2^- to form long-lived and highly cytotoxic peroxynitrite, which causes double-strand breaks in nuclear DNA. The researchers show that this MOL (named SNAP-MOL), when combined with radiotherapy, inhibits tumor growth in mouse models of colorectal and triple-negative breast cancer.

Nanoparticle-Mediated Toll-Like Receptor Activation and Dual Immune Checkpoint Downregulation for Potent Cancer Immunotherapy | *ACS Nano*, 2025 February 26

Nitric Oxide-Releasing Nanoscale Metal-Organic Layer Overcomes Hypoxia and Reactive Oxygen Species Diffusion Barriers to Enhance Cancer Radiotherapy | *Advanced Science*, 2025 January 1

How to make a cancer cell conspicuous

A paucity of targetable cancer antigens on many tumor types has long posed a challenge to immunotherapy. The trouble is not just that cancer cells adapt to be less detectable to patrolling immune cells but also that otherwise healthy cellular processes contribute to this low immunogenic profile. One such process, known as "nonsense-mediated decay" (NMD), destroys the transcripts of genes that are truncated by mutation so their products don't mess up the cellular works. Such mutant transcripts are common in cancer cells, whose genomes are riddled with mutations. If permitted expression, the aberrant proteins they encode could boost the number of cancer antigens presented on the cell surface by HLA molecules for immune detection. To get these suppressed transcripts translated, however, this quality control pathway needs to be blocked. Researchers led by Ludwig Johns Hopkins' Ashley Cook, Co-director Kenneth Kinzler and Nicolas Wyhs developed a cell-based screening assay to identify inhibitors of the NMD pathway to see if this might be possible. They reported in a February issue of *eLife* that an inhibitor of SMG1, an NMD pathway kinase, slows tumor growth in mice. The drug, KVS0001, boosts expression of truncated gene transcripts in human and murine cells *in vitro* and murine cells *in vivo*. It also increases the HLA class I presentation of such potential antigens, suggesting it could help boost the efficacy of cancer immunotherapies.

Identification of nonsense-mediated decay inhibitors that alter the tumor immune landscape
eLife, 2025 February 17

Ashley Cook

Kenneth Kinzler

Nicolas Wyhs

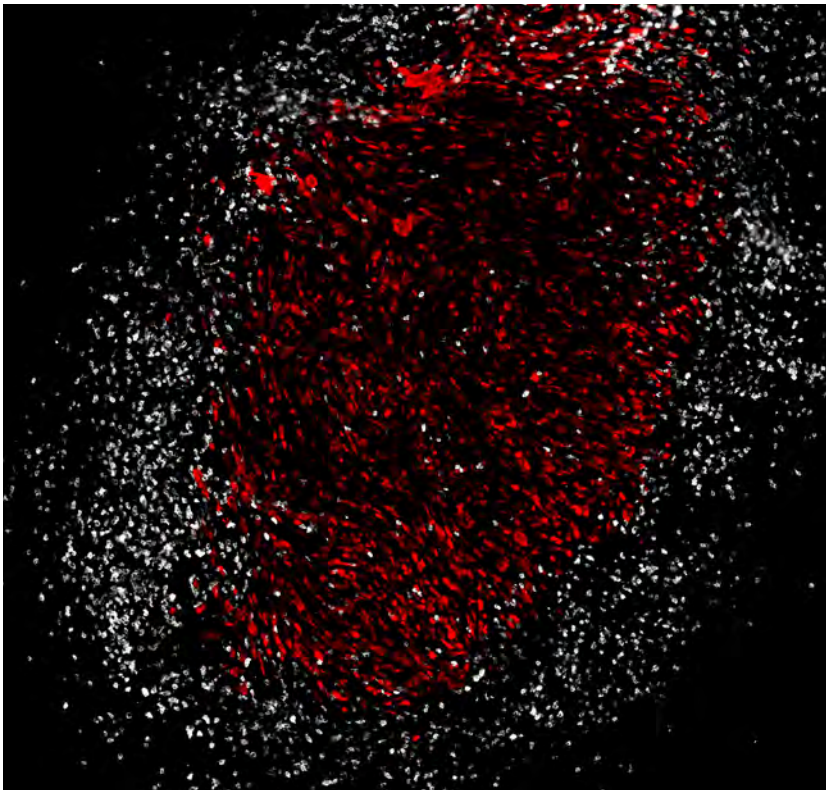
Marcia Haigis

A common and targetable metabolic dependency of lung cancers

About 4%-6% of lung adenocarcinomas, and a third of those diagnosed in people under 40 years of age, are driven by the anaplastic lymphoma kinase (ALK) gene fusion product. A continuously activated enzyme, ALK drives cancer cell proliferation through intracellular signaling mediated by Ras and its downstream effector MAP kinase. Lung cancers bearing this and analogous driver mutations are treated with tyrosine kinase inhibitors. But little was known about how the many metabolic adaptations of cancer cells contribute to sensitivity or resistance to such therapies. Researchers led by Ludwig Harvard's Marcia Haigis took a proteomics-based approach to address this question and identified GUK1, an enzyme that synthesizes GDP, as a metabolic vulnerability in patient-derived ALK+ cell lines. They reported in a February publication in *Cell* that ALK binds and phosphorylates GUK1 at tyrosine 74 (Y74) to boost GDP synthesis and amplify signaling by Ras and MAP kinase. Mutating that tyrosine (Y74F) reduces GDP and GTP pools in cells, inhibits Ras signaling—which depends on GTP—and MAP kinase activation, suppressing tumor cell proliferation in cultures and in mice. This suggests that inhibiting GUK1 activation could help treat ALK+ adenocarcinomas. Notably, Marcia and colleagues find that other oncogenic mutations in lung cancer also regulate GUK1, suggesting its activation could be a more general—and equally targetable—metabolic dependency of lung cancers.

GUK1 activation is a metabolic liability in lung cancer | *Cell*, 2025 February 6

Immunosuppression: a core issue for lung cancer immunotherapy



Elen Torres (Spranger Lab)

Stefani and her colleagues discovered a mechanism by which non-small cell lung cancers expressing SOX2 exclude effector T cells from the tumor core. Tumor cells expressing SOX2 are stained red, while adoptively transferred CD8 T cells are stained white.

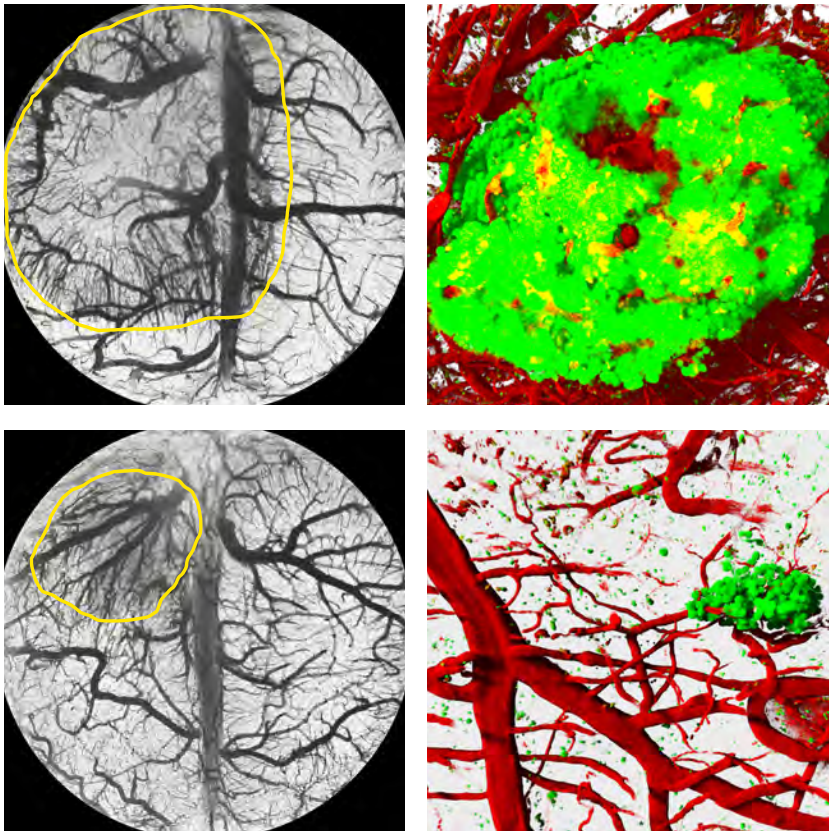
Stefani Spranger

Combination checkpoint blockade therapy (CBT) employing anti-CTLA-4 and anti-PD-1 antibodies has been shown to significantly improve overall survival of patients with non-small cell lung cancer (NSCLC). But the treatment only benefits about a third of all patients. This is mainly due to the many mechanisms by which tumor cells directly and indirectly suppress the anti-tumor immune responses engaged by CBT. These include signaling pathways activated within cancer cells that manipulate the immune landscape of the tumor's internal microenvironment. Researchers led by Ludwig MIT's Stefani Spranger reported in a January issue of *Cancer Immunology Research* a novel mechanism by which NSCLC tumors exclude effector T cells from their core. They found that expression of the developmental transcription factor SOX2 by NSCLC cells induces expression of the chemotactic factor CCL2 and boosts recruitment of regulatory T cells (Tregs) into the tumor that express the regulatory protein GITR at high levels on their surface. These Tregs alter the tumor vasculature to exclude CD8+ T cells from the tumor core, promoting resistance to CBT. Depleting GITR+ Tregs in mouse models improved CD8+ T cell infiltration of the tumor core and sensitized tumors to CBT, suggesting a strategy to improve NSCLC responses to immunotherapy.

Lung cancer-intrinsic SOX2 expression mediates resistance to checkpoint blockade therapy by inducing Treg cell-dependent CD8+ T-cell exclusion
Cancer Immunology Research, 2025 January 2

An approved drug could activate anti-tumor immunity to treat a childhood brain cancer

Molecular profiling has identified various subtypes of the brain cancer ependymoma (EPN), including a supratentorial EPN (ST-EPN) driven by the oncogenes ZFTA–RELA and EPHB2. But little is known about the immune system's role in EPN progression and response to therapy. Researchers led by Ludwig Harvard's Rakesh Jain used an immunocompetent mouse model of ST-EPN-ZFTA to explore this aspect of EPN and identify potential drug targets for the cancer. Gene-expression profiling of these tumors, and analysis of a human EPN tumor dataset, revealed several protein kinases that could be targeted for therapy. Building on these findings, the researchers found that dasatinib, an FDA-approved leukemia drug with broad-spectrum inhibition of protein kinases, suppressed EPN growth in culture and in mice. They reported in a January publication in *PNAS* that dasatinib potently inhibits the growth of this type of EPN in their mouse models. The inhibitor, they discovered, reprogrammed pro-tumor tumor-associated macrophages (TAMs) into an anti-tumor state and boosted the activation of cytotoxic CD8+ T cells that kill cancer cells. The treatment induced complete regression of established EPN tumors of this subtype in 78% of the mice and prevented tumor recurrence. Depleting T cells abrogated the therapeutic effect, showing anti-tumor immunity is central to the tumor regression induced by dasatinib.



Jun Ren

Intravital optical coherence tomography (OCT) imaging to detect perfused vessels (black) shows that vehicle-treated tumor had reduced blood perfusion in the center of tumor and increased angiogenesis (top left) while dasatinib treatment reduced tumor size and improved blood perfusion (bottom left). The yellow line marks tumor region.

Intravital multi-photon microscopy (MPM) shows, at single-cell resolution, that compared to vehicle-treated tumor (top right), dasatinib treatment reduced tumor size (bottom right) (Green: ependymoma tumor cells; Red: blood vessels).

Rakesh Jain

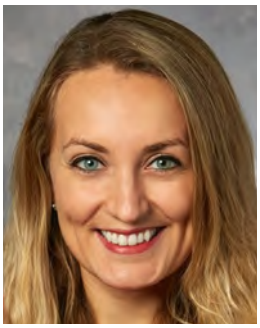
Targeting EPHB2/ABL1 restores antitumor immunity in preclinical models of ependymoma | *PNAS*, 2025 January 22

ELECTRIC CARs for AML therapy?

Treatment-refractory or relapsed acute myelogenous leukemia (AML) is treated with allogeneic hematopoietic stem cell transplantation (HSCT). In allo-HSCT, the patient's HSCs and leukemic stem cells (LSCs) are both eliminated with high-dose genotoxic chemotherapy and irradiation in a "conditioning" regimen before HSCT. But nearly a quarter of children with AML relapse following HSCT, and half succumb to disease or to treatment-related morbidities within 5 years. Researchers led by Ludwig Stanford's Quenton Bubb and Agnieszka Czechowicz reported in a January paper in *Molecular Therapy Oncology* proof of concept for a new tool to reduce the morbidity stemming from HSCT itself. Their strategy employs novel chimeric antigen receptor (CAR)-T cells for both anti-leukemic therapy and HSCT conditioning. The cells are engineered with CARs that, uniquely, simultaneously target three cytokine receptors (KIT, MPL and FLT3) expressed by both LSCs and healthy HSCs. These "extracellularly linked concatemeric trivalent cytokine" (ELECTRIC) CAR-T cells, they propose, would eliminate the need for chemotherapy or irradiation in the conditioning regimen. Quenton, Agnieszka and colleagues showed that ELECTRIC CARs exhibit potent cytotoxicity against normal and malignant hematopoietic cells *in vitro* and display activity against cells bearing the targeted cytokine receptors in a murine xenograft model.

Development of multivalent CAR T cells as dual immunotherapy and conditioning agents
Molecular Therapy Oncology, 2025 January 30

Quenton Bubb



Agnieszka Czechowicz

Kornelia Polyak

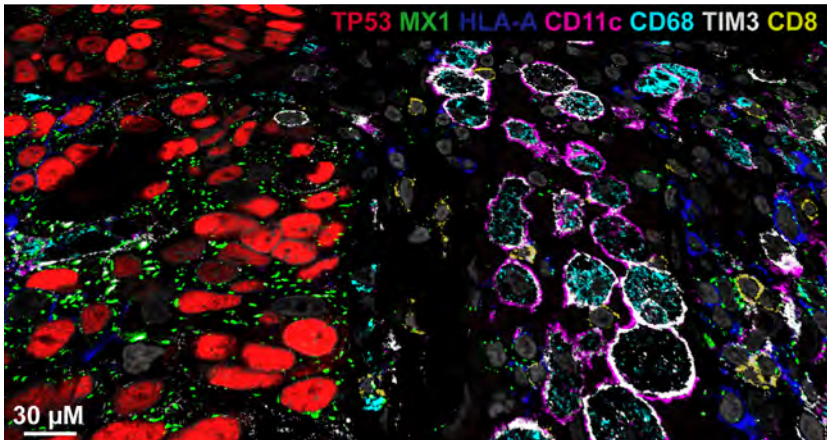
An epigenetic enzyme's role in basal breast cancers

Gene expression analysis of breast cancers has discerned three molecular subtypes—luminal, basal, and mesenchymal—across the classically defined (ER+, HER2+ and triple-negative, or TNBC) types of the cancer. Ludwig Harvard's Kornelia Polyak and her colleagues have previously identified epigenetic enzyme KDM5B, a histone demethylase, as a luminal lineage-driving oncogene frequently amplified in ER+ breast tumors that also contributes to endocrine therapy resistance. That oncogene's paralog, KDM5A, appears to play a similar and redundant role in ER+ tumors. In a December issue of *Cell Reports*, Kornelia and her team described their findings from a follow-up study exploring KDM5A's role in basal and triple-negative breast cancers. They reported that KDM5A is specifically amplified and overexpressed in basal breast tumors and its inhibition suppresses the growth of a *KDM5A*-amplified basal TNBC cell line. The researchers used this cell line to perform CRISPR screens to identify proteins that modulate sensitivity to the inhibitor. They discovered that deletion of the *ZBTB7A* transcription factor and core SAGA complex—a multi-subunit, enzymatic coactivator of transcription—sensitizes cells to KDM5 inhibition, while deletion of RHO-GTPases confers resistance to the KDM5 inhibitor. The researchers showed that KDM5A binding depends on ZBTB7A and described the molecular mechanisms associated with this effect. They also reported that high *ZBTB7A* expression is associated with poor response to neoadjuvant chemotherapy in triple-negative breast cancer.

ZBTB7A is a modulator of KDM5-driven transcriptional networks in basal breast cancer
Cell Reports, 2024 December 24

Clues to catching a deadly cancer early

The molecular changes that occur as precancerous lesions progress to high-grade serous ovarian cancer (HGSOC) are not well characterized. With an overall 5-year survival rate of just 30%, the cancer originates in the fallopian tubes (not the ovaries) and has two recognized precursor lesions: p53 signatures (benign-looking secretory cells that sport mutations in the tumor suppressor *TP53*) and serous tubal intraepithelial carcinomas (STIC). Looking for features that might help catch HGSOC early, researchers led by Ludwig Harvard's Sandro Santagata, Peter Sorger, Tanjina Kader, Jia-Ren Lin and Clemens Hug integrated a high-plex imaging platform, CyCIF, and spatial transcriptomics to analyze human samples from progressive stages of HGSOC development, including p53 signatures, STICs and invasive disease. They described in an online publication in *Cancer Discovery* in December some common features of precursor epithelial cells, including persistent interferon (IFN) signaling, chromosomal instability and aberrant innate and adaptive immunity. STIC epithelium had relatively high expression of MHC-I molecules and IFN-stimulated genes that are common features of late-stage tumorigenesis. These alterations coincide with progressive shifts in the tumor microenvironment, transitioning from immune surveillance in early STICs to immune suppression in advanced STICs and cancer. Their findings identify molecular markers that could be useful for HGSOC diagnostics and provide clues for developing therapies to treat earlier and more medically tractable stages of HGSOC progression. The data is available for public exploration in cBioPortal.



Tanjina Kader

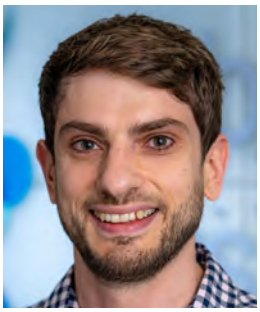
Multiplex imaging of a high-grade serous ovarian carcinoma (TP53 mutant) reveals tumor cells overexpressing interferon response markers (MX1$^+$, HLA-A$^+$) in close association with TIM3$^+$ antigen-presenting cells (CD11c$^+$CD68$^+$), or APCs. The image highlights a potentially immunosuppressive microenvironment, where APCs may have reduced capacity to activate CD4$^+$ and CD8$^+$ T cells despite their abundance.

Sandro Santagata

Peter Sorger

Tanjina Kader

Jia-Ren Lin



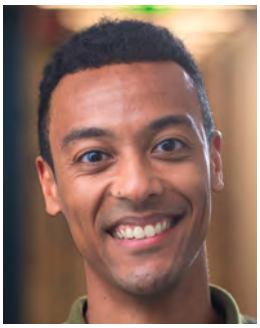
Clemens Hug

Multimodal Spatial Profiling Reveals Immune Suppression and Microenvironment Remodeling in Fallopian Tube Precursors to High-Grade Serous Ovarian Carcinoma | *Cancer Discovery*, 2024 December 20

Clues to the earliest signs of impending breast cancer

Women carrying mutant BRCA1 and BRCA2 genes are at higher risk for several cancers. There is thus a significant need to identify molecular markers to prevent or catch cancer early in such people. To address this need, researchers co-led by Ludwig Harvard's Michael Oliphant and Co-director Joan Brugge with colleagues at other institutions explored chromosomal copy number alterations (CNAs) in more than 48,000 individual breast cells isolated from noncancerous breast tissues of 28 women with both high-risk *BRCA1/BRCA2* germline mutations or with unaltered BRCA genes. In the November issue of *Nature Genetics* they reported that almost all women had a small subset of breast cells (about 3%) that harbored one to four specific CNAs commonly found in cancerous tissues. These CNAs—which arise in BRCA1/2 mutant carriers prior to the loss of the remaining normal copy of the genes (loss of heterozygosity), a key step in cancer progression—were found exclusively in luminal cells that line breast ducts, the presumed origin of most breast cancers. In a few women with high risk BRCA1/2 mutations, the researchers identified cells that carry an additional set of CNAs, including deletion of the normal copy of BRCA1 or BRCA2—presumably cells on their way to breast cancer. The findings suggest these rare aberrations in luminal cells may be some of the earliest events in the evolution of malignant breast tumors.

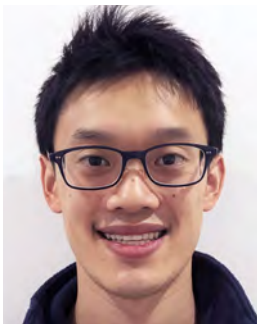
Luminal breast epithelial cells of BRCA1 or BRCA2 mutation carriers and noncarriers harbor common breast cancer copy number alterations | *Nature Genetics*, 2024 November 20



Michael Oliphant

Joan Brugge

Yang Shi

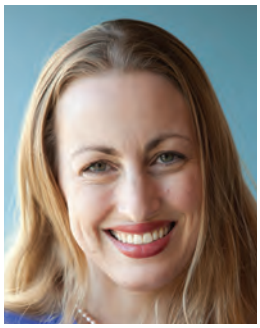


Alan Jiao

How a histone mutation drives a pediatric brain cancer

Some 80% of diffuse midline gliomas (DMGs)—a family of swiftly lethal pediatric brain cancers—are driven by the H3K27M histone mutation. How precisely this drives cancer is not entirely clear. Components of chromosomes, histones package and organize DNA in the nucleus and chemical, or "epigenetic", modifications made to them help regulate gene expression. One effect of the H3K27M mutation is the global loss of H3K27 trimethylation, which is an epigenetic modification associated with gene silencing. Researchers led by Ludwig Oxford's Yang Shi and Alan Jiao used a genetic screen in *C. elegans* to identify ways to potentially reverse the abrogation of H3K27 trimethylation. They reported in a November *PNAS* paper 20 suppressor mutations that to varying degrees reverse that effect. While 19 of them mapped to the H3K27M mutant itself, one suppressive mutation, surprisingly, was to the E2 ubiquitin-conjugating enzyme *ubc-20*. Loss of *ubc-20* activity restored H3K27Me3 to nearly half that observed in normal cells. The researchers show that *ubc-20* is responsible for generating diubiquitinated histone H2B, a previously unstudied modification. This suggests the effects of H3K27M may be modulated by H2B diubiquitination and that aberrations in such modifications might regulate the oncogenic effects of histones, though *ubc-20* may have other substrates whose modifications could also impact K27M function. The discovery opens a door to new strategies for treating H3K27M-driven DMGs.

An E2 ubiquitin-conjugating enzyme links diubiquitinated H2B to H3K27M oncohistone function | *PNAS*, 2024 November 19



Michelle Monje

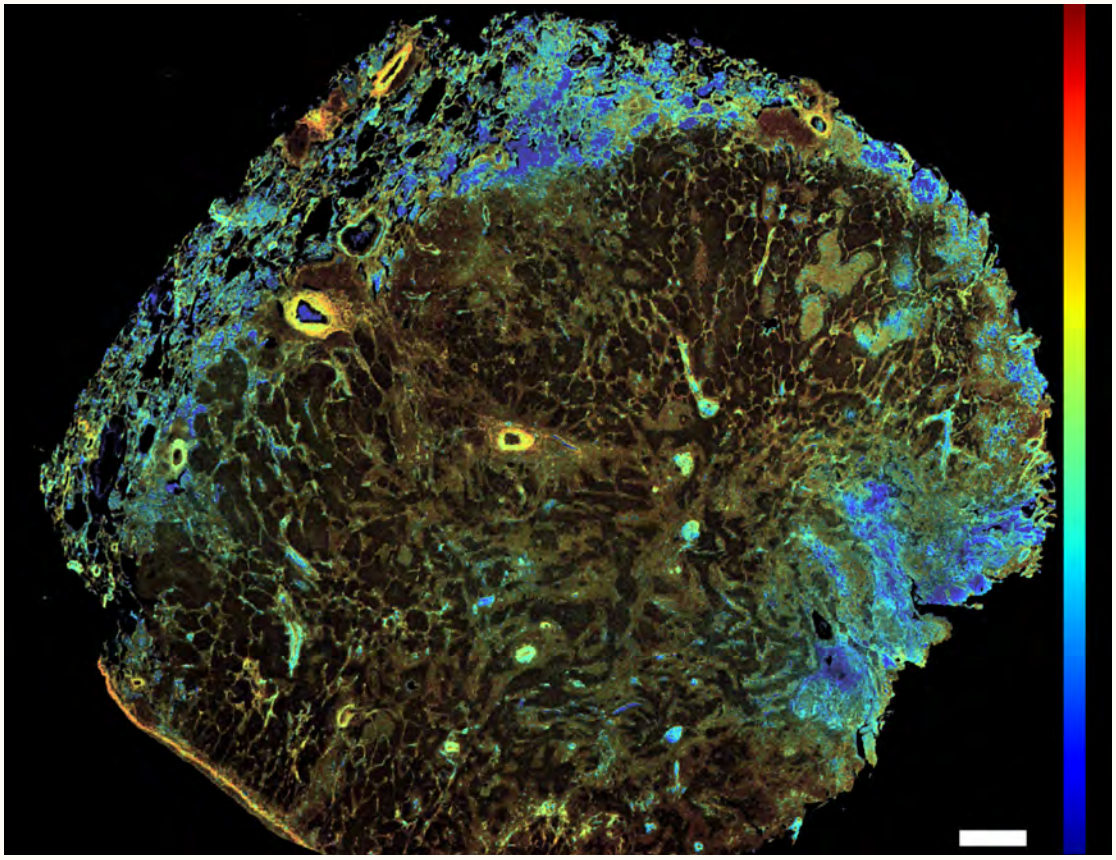
Crystal Mackall

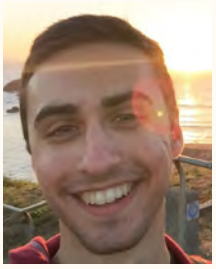
CAR-T therapy delivers remarkable results against a pediatric brain cancer

Tumors of the brain and spinal cord, H3K27M-mutated diffuse midline gliomas (DMGs), including diffuse intrinsic pontine gliomas (DIPG), occur in children and young adults and have a median overall survival of 11-13 months from diagnosis. Palliative radiotherapy is currently the standard of care for these cancers, which cannot be surgically excised and are incurable. Researchers led by Ludwig Stanford's Michelle Monje and Crystal Mackall reported in *Nature* last November the final results for Arm A of an ongoing Phase 1 trial evaluating the use of CAR-T cells directed against the ganglioside GD2—which the researchers previously had shown to be highly expressed in H3K27M+ DMGs—for these cancers. Thirteen patients with spinal DMG (3) and DIPG (10) enrolled in the trial, with eleven treated on trial, receiving CAR-T therapy intravenously. Nine patients received subsequent intracranial CAR-T infusions, which provoked fewer side effects. (Two patients progressed too rapidly to receive the experimental therapy.) Associated neuroinflammation was both expected and clinically manageable. Remarkably, nine patients showed neurological improvement, while tumors in three patients shrank by more than half (52, 54 and 91%) with a further three patients exhibiting smaller reductions. One patient exhibited a complete response—with tumors undetectable in brain scans—that was ongoing for over 30 months since enrollment. This is the first such response recorded for a cancer that has resisted all other treatment strategies.

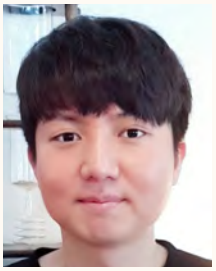
Intravenous and intracranial GD2-CAR T cells for H3K27M+ diffuse midline gliomas | *Nature*, 2024 November 13

Image gallery





Jackson Riseman



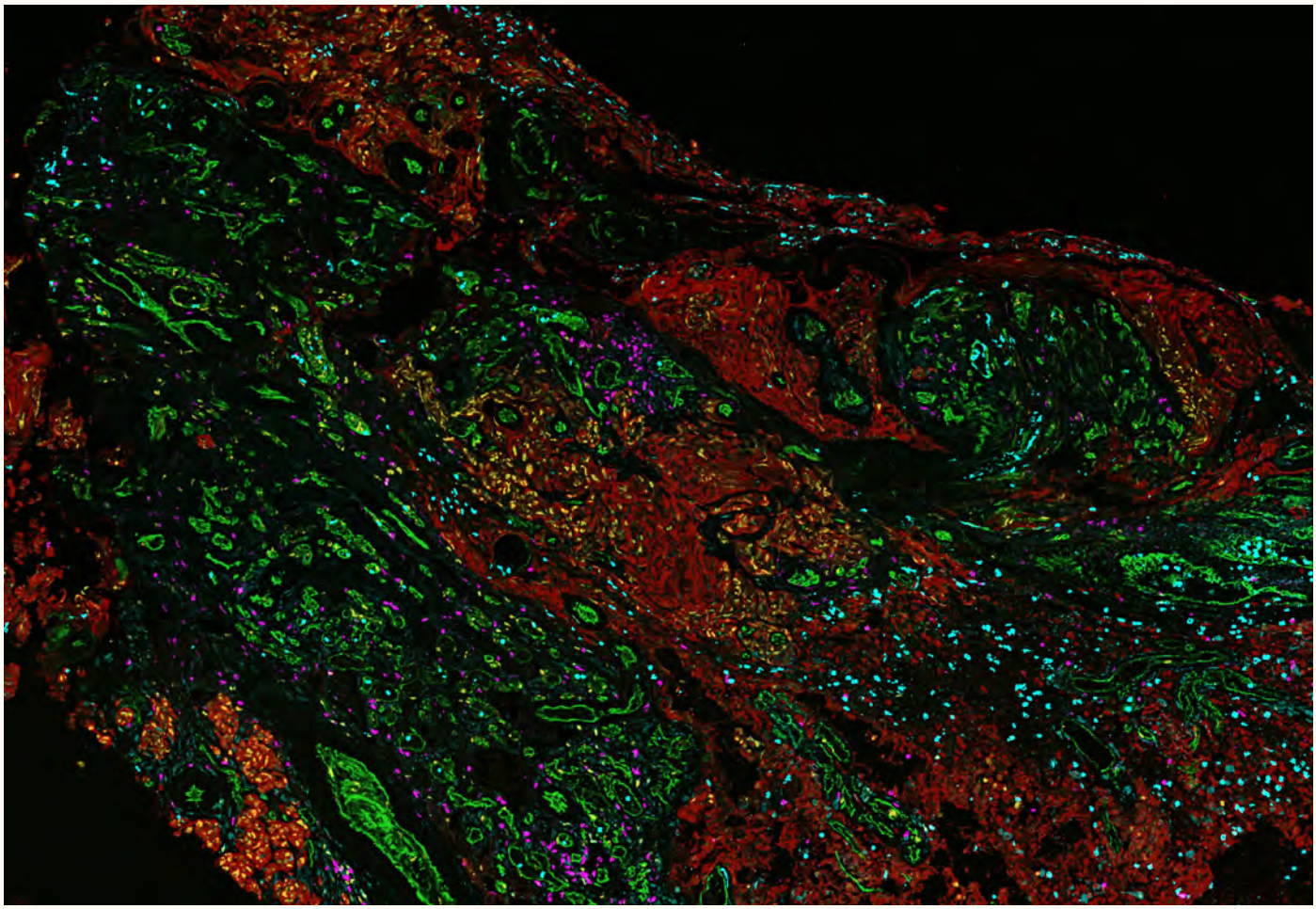
Wonsang Hwang

Conor Evans

This image was shared by Jackson Riseman, Wonsang Hwang, and Conor Evans at Ludwig Harvard.

"We captured this image when we were developing a label-free fluorescence lifetime imaging (FLIM) protocol designed specifically for routine formalin-fixed paraffin-embedded (FFPE) tissue sections, aiming to map the metabolic states within solid tumors," Conor told us. The image captures NADH signals from an FFPE lung carcinoma section. Blue regions indicate glycolysis. Red regions signify oxidative phosphorylation. By measuring the intrinsic fluorescence lifetime of NADH, the method distinguishes areas dominated by glycolysis (short-lived, free NADH) from those relying on oxidative phosphorylation (long-lived, protein-bound NADH). This image is a whole-section overview obtained during method validation, demonstrating that a single-slice FLIM measurement effectively preserves histological context while clearly visualizing the spatial heterogeneity of tumor metabolism. "Our research is still at an early stage, and there is much yet to uncover about the precise implications of these observed metabolic patterns, so I'm cautious about interpreting this data," Conor said. "But I personally find this image interesting because it appears to reveal spatial features of the tumor microenvironment that, to my knowledge, have not previously been reported. The lifetime map clearly exposes metabolic heterogeneity, not only within the tumor core but also in the surrounding tissue that appears histologically normal."

The jet color map ranges from 1 ns (blue) to 3 ns (red). Scale bar: 2 mm.



This image was shared by Kornelia Polyak, Jun Nishida and Kun Huang at Ludwig Harvard.

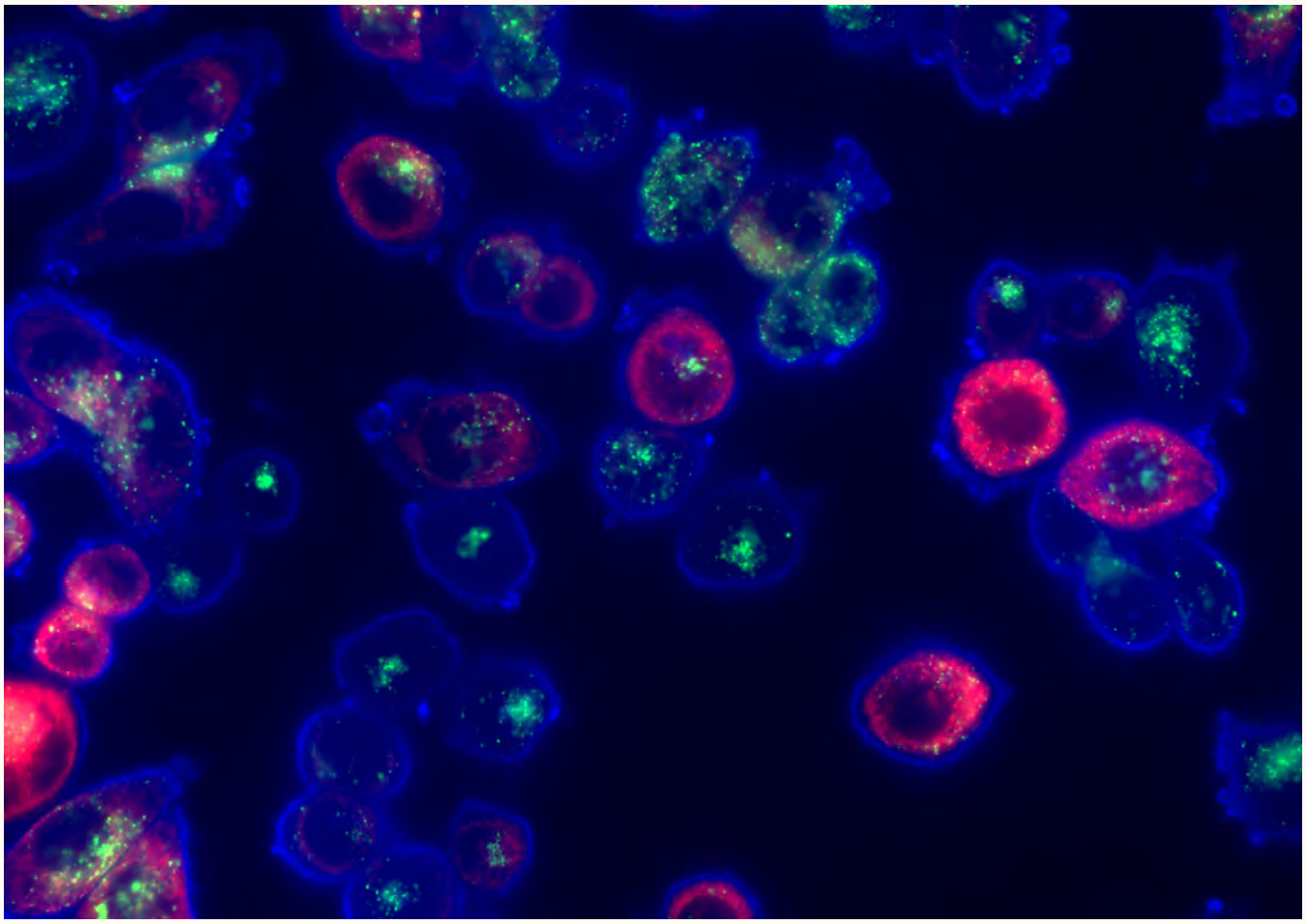
"Breast cancer brain metastasis is a devastating disease with limited treatment options," Kornelia told us. "We have been characterizing the spatial heterogeneity of breast cancer brain metastases with the aim of better understanding how cancer cells adapt to grow in the tissue microenvironment of the brain, and how we might be able to target this process to treat brain metastases. This is a phenocycler image visualizing immune cells (magenta, cyan) in a brain metastasis of breast cancer (cancer cells, red). It shows how immune cells accumulate at the interface of the tumor and its stroma. Research on brain metastasis holds a special meaning for Jun Nishida, the fellow leading this project, as he lost a family member to this disease."

Kornelia Polyak

Jun Nishida

Kun Huang

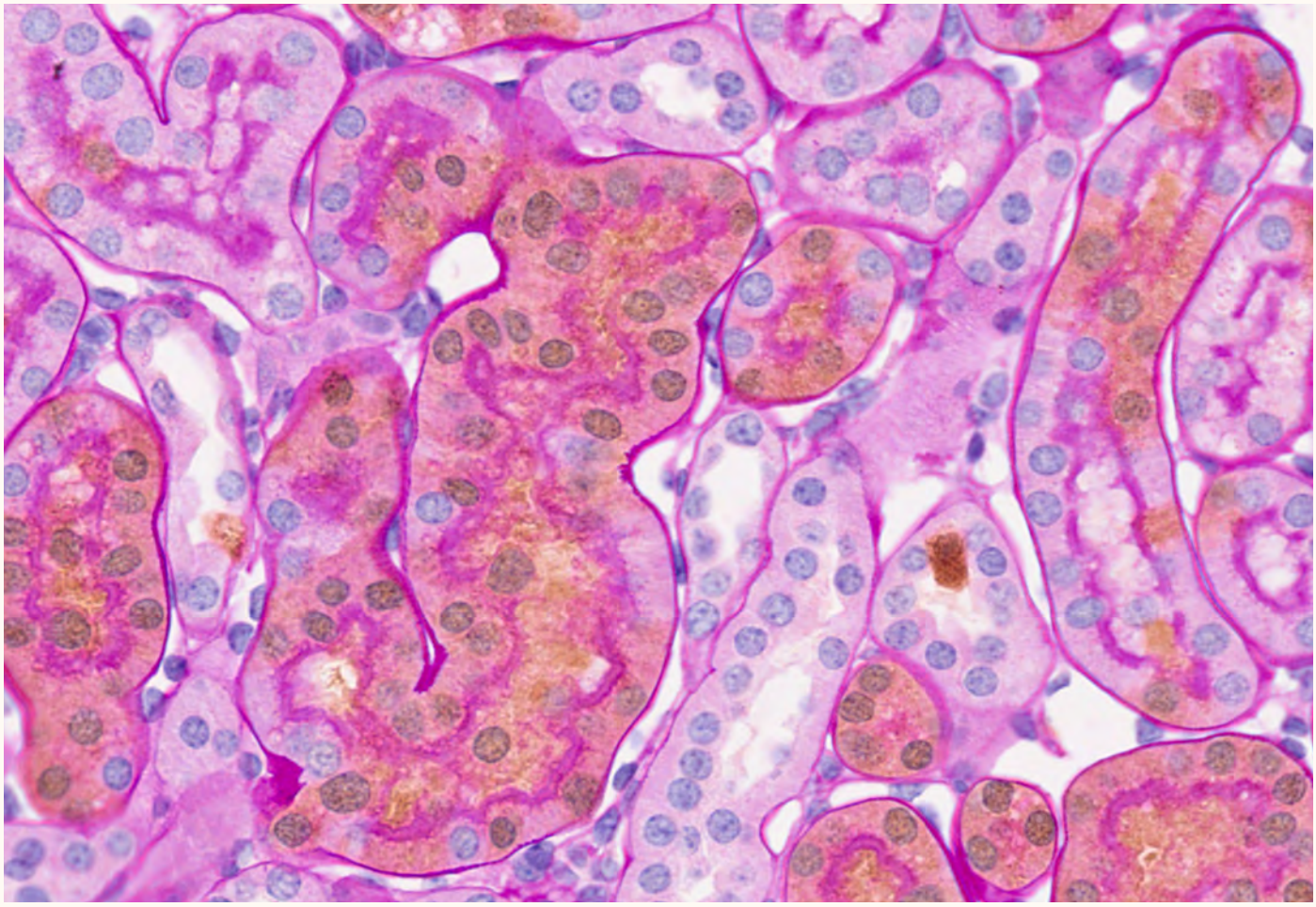
Image gallery



Alex Toker

This image was shared by Alex Toker at Ludwig Harvard.

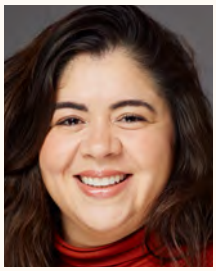
The image (which looks to the Link a little like something from the Hubble Space Telescope) shows MDA-MB-468 triple negative breast cancer (TNBC) cells growing *in vitro*, stained with markers to detect cellular localization of cholesterol. It stems from a study reported last year by Ludwig Harvard's Alex Toker and alumnus Alissandra Hillis, who found that treatment with AKT inhibitors makes TNBC cells exceptionally vulnerable to disruptions in their cholesterol balance (homeostasis) induced by a statin drug (pitavastatin). Combining an FDA-approved AKT inhibitor with pitavastatin kills TNBC cells in patient-derived estrogen receptor (ER)–negative breast cancer organoids and impairs tumor growth and decreases tumor size in mice bearing xenografts of TNBC tumors. The blue channel is Filipin III which stains cholesterol, the green channel is LAMP1, a lysosomal marker, and the red channel is an endoplasmic reticulum marker using a red fluorescent protein tagged expression construct. The staging pattern shows a predominantly plasma membrane cholesterol localization at baseline.



This image was shared by Samvid Kurlekar and Joanna Lima, postdocs in Peter Ratcliffe's lab at Ludwig Oxford.

"Our lab studies the earliest events of oncogenesis in the kidneys following von Hippel Lindau (VHL) and Polybromo-1 (Pbrm1) gene inactivation in the renal cortical tubular epithelium," Samvid and Joanna said. "We trace the behavior of early oncogenic cells using a cell tagging mouse model that labels Vhl/Pbrm1-null cells with a tdTomato reporter. We have developed a dual immunohistochemistry (IHC)/Periodic Acid Schiff (PAS) staining protocol that allows us to identify and assay Vhl/Pbrm1-null cells (stained 'brown' by tdTomato IHC) against the structure of renal cortical tubules, whose basement membranes and brush borders are stained pink with PAS. In this image, we can identify specific Vhl/Pbrm1-null cells with altered morphology that have either extruded into the tubular lumen or are invading the renal interstitium.

Samvid Kurlekar

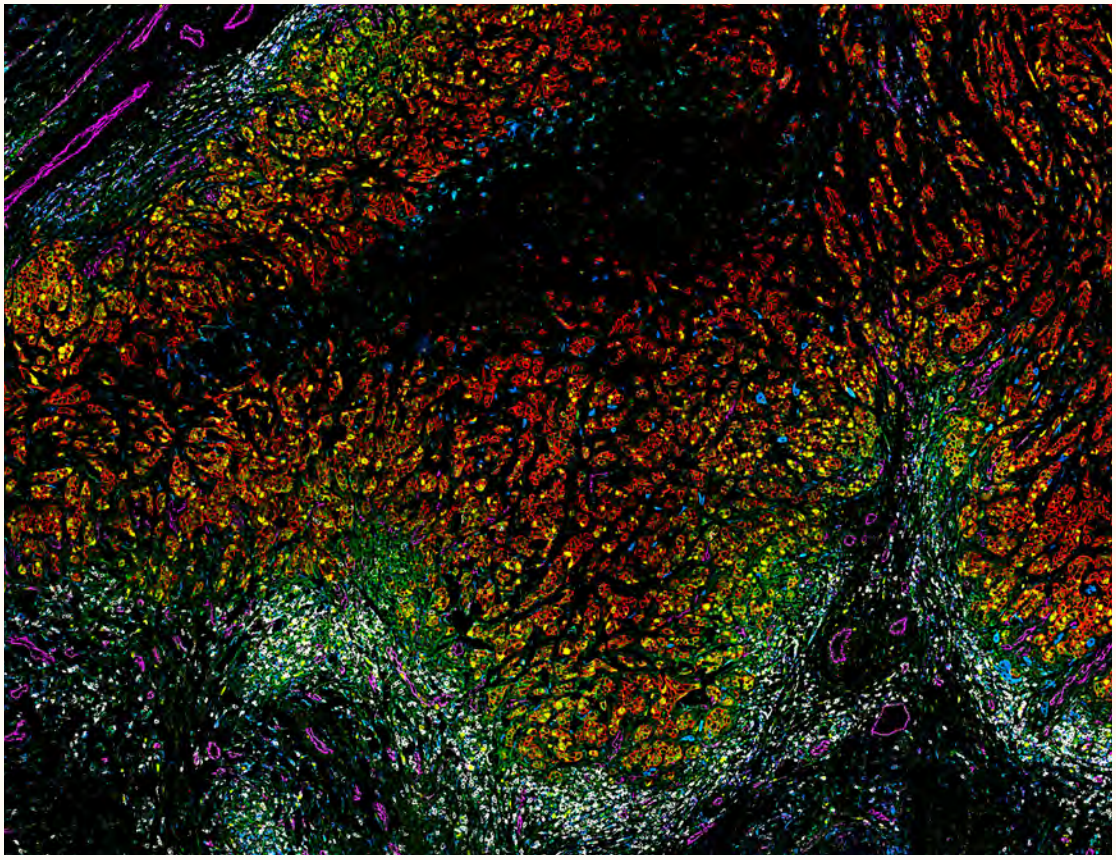


Joanna Lima

Peter Ratcliffe

This invasive behavior is potentially of great relevance to oncogenesis and merits further study. In addition to its scientific and aesthetic value, this image shows us that in an age of ever-expanding "omics" technologies, profound biological discoveries can still be made with decades-old IHC and PAS technologies."

Image gallery



Ye Tian

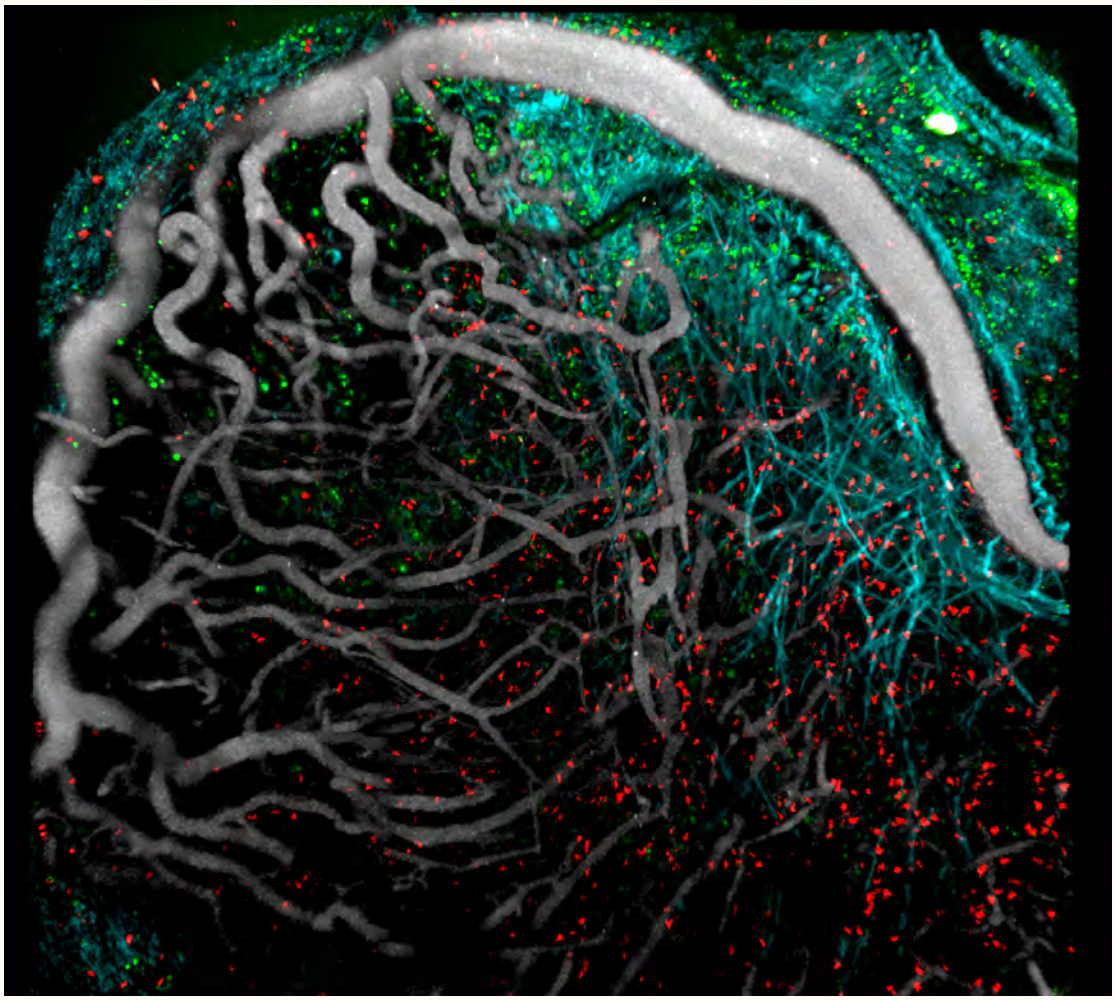
Maxime Meylan

Kai Wucherpfennig

This image was shared by Ye Tian, Maxime Meylan and Kai Wucherpfennig at Ludwig Harvard.

This image comes from an ongoing study investigating the spatial features that drive immune exclusion in human triple-negative breast cancer (TNBC) tumors. "In our preliminary work, we found that the T cell infiltration in human TNBCs is correlated with tumor spatial patterns, which are highly heterogeneous within and between tumors," Ye Tian said. "Taken in 2022, the image exhibits a typical immune-excluded pattern of TNBC, in which the T cells accumulate around the tumor edge but are unable to infiltrate into the tumor nest. The sample imaged here is an FFPE section of a human TNBC tumor imaged using the CODEX multiplex imaging platform equipped with a Zeiss Axio Observer 7 microscope.

The image highlights the immune-exclusion spatial feature of this TNBC case. Tumor cells (E-Cadherin$^+$, red) are highly proliferative (Ki67$^+$, yellow) and exhibit heterogeneous MHC-I expression. Macrophages (CD68$^+$, blue) and blood vessels (CD31$^+$, magenta) infiltrate the tumor, while numerous cytotoxic T cells (CD8$^+$, white) are restricted to the tumor periphery and excluded from the tumor nest. I love the color pattern of this beautiful micrograph, which simultaneously and clearly depicts six parameters of the tumor microenvironment. Our title for the image, *Siege and Stalemate*, not only captures the local pattern between tumor cells and T cells in this particular sample but also refers to the broader impasse between TNBC and currently available immunotherapies: For a substantial proportion of TNBC patients, immunotherapies have yet to achieve durable and effective responses."



Stefania Vilbois

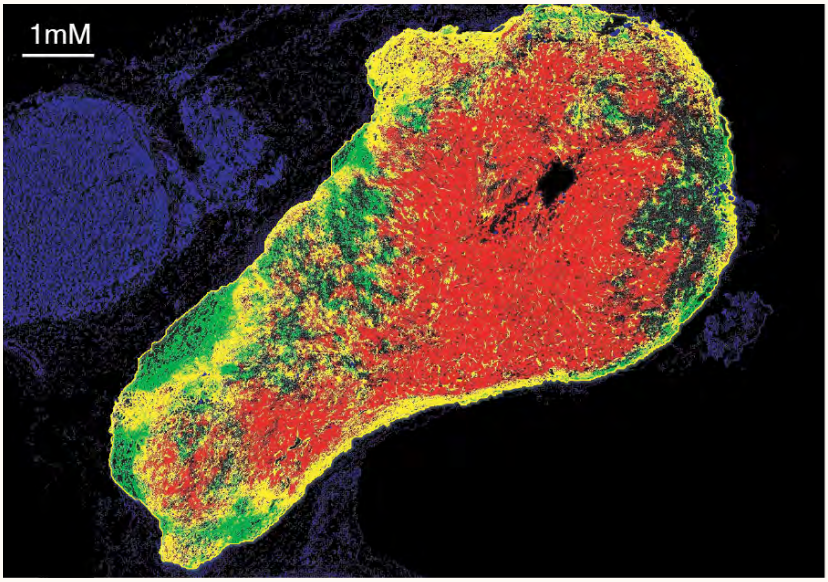
Ping-Chih Ho

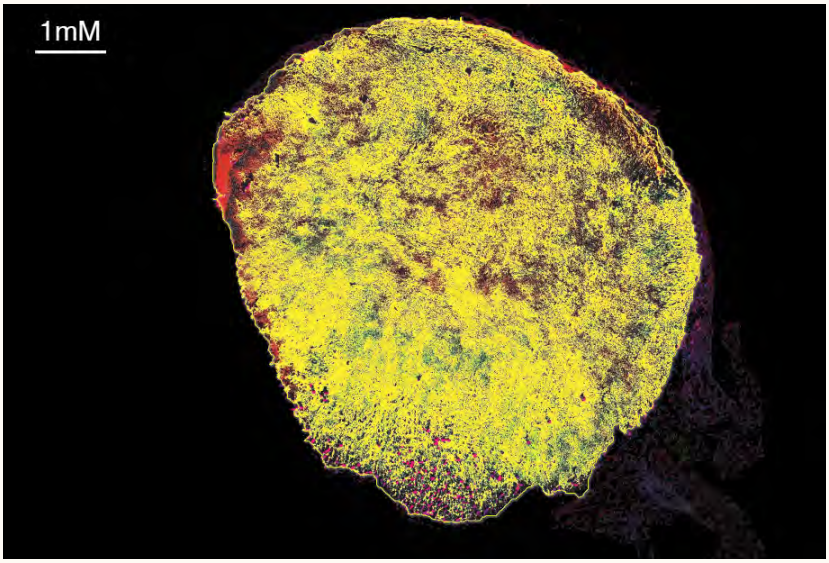
This image was shared by Stefania Vilbois, a graduate student in Ping-Chih Ho's laboratory at Ludwig Lausanne.

This is an intravital 2-photon image of a mouse melanoma ten days post-engraftment, with CD8+ tumor-infiltrating lymphocytes (TILs, orange and red), dendritic cells (green), blood vessels (Qdot, gray), and collagen fibers (second harmonic generation, cyan).

"To track the localization of tumor-specific CD8+ T cells and conventional type 1 dendritic cells (cDC1) in the tumor microenvironment (TME), we used a mouse reporter model (XCR1-DTR-Venus) to monitor cDC1 with a Venus reporter (FITC, green)," Stefania told us. "We adoptively transferred naive tumor-specific CD8+ T cells from a mouse cell lineage tracing strain before tumor engraftment. Using this cell lineage tracing system, we identified TCF1+ CD8+ progenitor T cells (GFP and Tdtomato, orange) that differentiated into TCF1- CD8+ exhausted T cells (Tdtomato, red). We are investigating the role of cDC1 in the tumor microenvironment and how it can improve the anti-tumor immune response. One way is by preserving the progenitor-like TCF1+ state of CD8+ TILs. Another is by helping TILs to migrate into the tumor. We compared the distribution of progenitor-like and exhausted-like TILs in the presence of XCR1+ cDC1 and after XCR1+ cDC1 depletion. The image was taken in July 2021. It was my first session with the 2-photon microscope, and we were fortunate that the setup worked in the first try. Unfortunately, this wasn't always the case, as each mouse tumor grows differently, and stabilizing the tumor for long acquisition without being affected by the mouse breathing is challenging."

Image gallery





This image was shared by Jingjing Zhu, who is in Benoît Van den Eynde's laboratory in Brussels.

These images reveal the limited tumor penetration of systemically administered anti-PD-L1 antibodies and the preclinical success of a strategy to overcome this limitation by engineering tumor-targeting CD8+ T cells to locally secrete an anti-PD-L1 nanobody, leveraging the tumor-homing ability of the T cells. The approach has two potential advantages: Nanobodies are produced where needed, bypassing penetration issues, and their short half-life and localized delivery prevents systemic buildup, reducing the risk of side effects. "We showed that our strategy achieved superior tumor control in a mouse model compared to conventional anti-PD-L1 antibody injection," Jingjing told us. This improvement was primarily due to the improved tumor penetration of locally delivered nanobodies (bottom image, green)—which infiltrated the tumor and blocked PD-L1 (red) at the tumor site—compared to systemic anti-PD-L1 antibodies (top image, green). The yellow staining represents successful binding events (green + red = yellow), where the green-labeled antibodies have bound to red-labeled PD-L1 molecules in the tumor tissue. "The predominance of yellow in the [bottom] image suggests our engineered T-cell approach achieves superior target engagement compared to conventional antibody delivery (top panel), visually confirming our hypotheses."

Jingjing Zhu

Benoît Van den Eynde

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