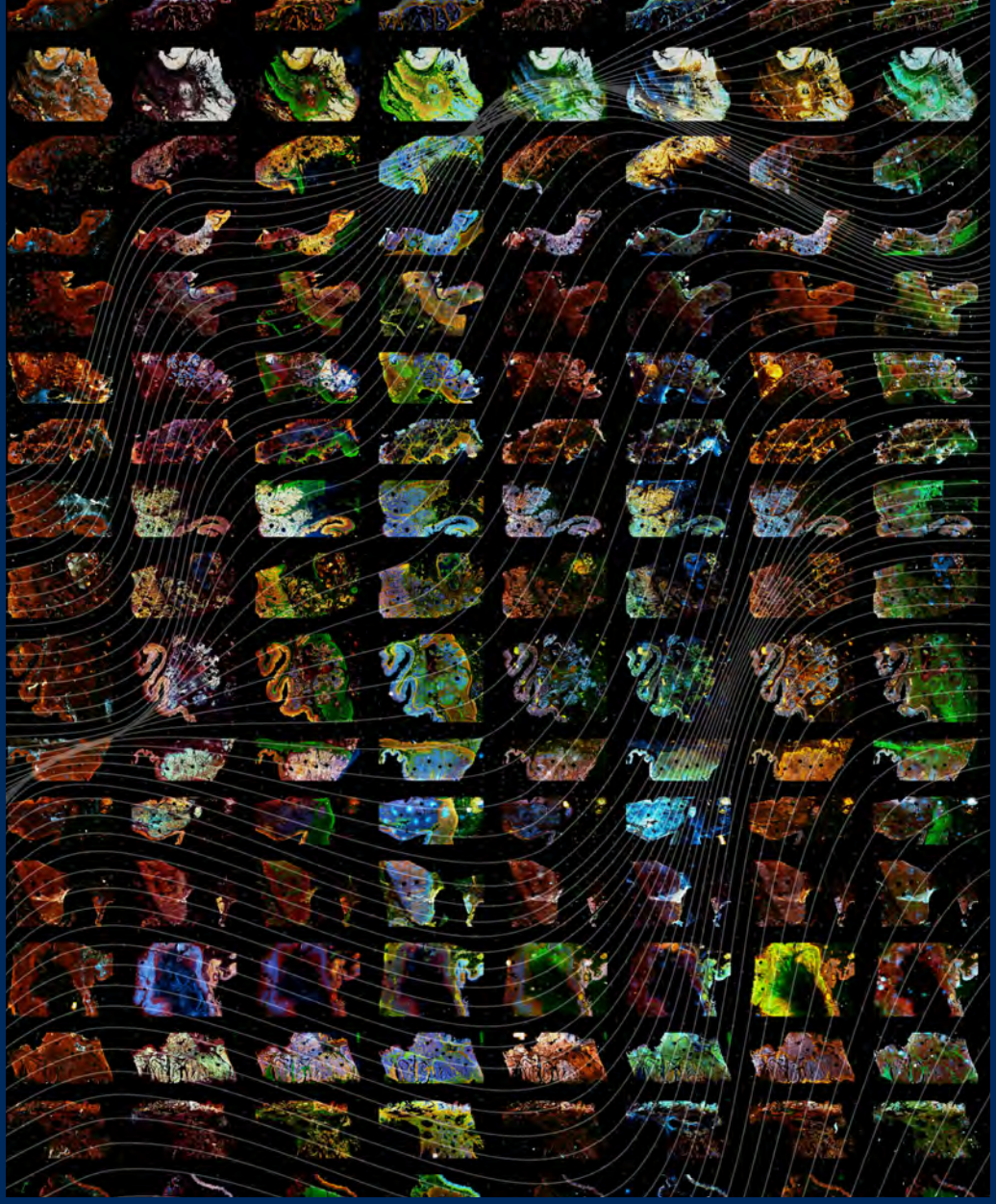


June 2023

Ludwig Link

LUDWIG CANCER RESEARCH

LIFE-CHANGING SCIENCE

Welcome

Unmesh Kher
Editorial Director

Welcome to the Summer 2023 issue of the Ludwig Link!

You might notice, first, that the photo accompanying this letter has changed. This is because our former Senior Vice President for Communications, Rachel Reinhardt—whose photo used to be there—has left for sunnier climes, specifically, Arizona (for more, see page 6). We promise to maintain the high standards she established in this and all future issues.

You'll still get, for starters, the rich assortment of discoveries made in Ludwig laboratories in our Research news section. We have features on how cancer cells may be reprogrammed into living cancer vaccines and how the immune system's surveillance of cancer can itself induce metabolic adaptations in tumors that simultaneously promote their growth and equip them to suppress immune responses. And that's just a sample of what's on the menu. Read on for more.

We also, sadly, bring news of deaths in the Ludwig community, namely those of Ludwig New York alumnus Herbert Oettgen, former Ludwig Lausanne Director Hugh Robson MacDonald and former Scientific Advisor Christopher Walsh. We offer our condolences to their loved ones and recall, with gratitude, their contributions to our organization and to cancer research.

Our Q&A in this issue (page 25) is with Ludwig Lausanne's Michal Bassani-Sternberg, who helped pioneer the field of immunopeptidomics and is applying it to aid the development of personalized immunotherapies. And, of course, we also have news of awards, promotions and honors earned by Ludwig researchers (page 5), and of Ludwig's participation in the AACR Annual Meeting. In our Ask a scientist section, Ludwig researchers tell us which area of cancer research they believe holds the greatest promise for someone now starting up a career in science. See what your colleagues said on page 30.

Happy reading!

Sincerely,

Unmesh Kher
Editorial Director

On the cover

A spread of images showing the 18 colorectal tumor samples used by a Ludwig Harvard team led by Peter Sorger and Sandro Santagata to generate 2D and 3D maps of the cancerous tissue. The researchers applied highly multiplexed tissue imaging, 3D reconstruction methods, spatial statistics and machine learning to identify cell types and states underlying morphological features of known diagnostic and prognostic significance in colorectal cancer. Their maps revealed, among other things, key immune interactions at the invasive boundary of tumors and previously unknown interconnections between histological structures. Image by Yu-An Chen, Sorger lab.

STORY ON PAGE 22

In this issue

"We are being challenged to do things very robustly, to do things in a very, very careful way, but also to innovate and challenge ourselves with scientific questions that are linked to the burning questions and needs of the clinic."

Q&A with Michal Bassani-Sternberg, page 25

FEATURED RESEARCH

Teams led by Ludwig Lausanne's Mikaël Pittet and, separately, Ludwig Weill Cornell's Taha Merghoub and Jedd Wolchok found that neutrophils—long considered abettors of cancer growth—are key to the success of immunotherapy. **Page 14**

Whether you're a young scientist or a senior researcher, which area of cancer research do you believe holds the greatest promise for someone now starting up a career in science?

Ask a scientist, page 30

George Coukos

Jedd Wolchok

Ludwig Lausanne's George Coukos and Ludwig Weill Cornell's Jedd Wolchok elected Fellows of the AACR

Ludwig Lausanne Director George Coukos and Jedd Wolchok, who is co-director of the Ludwig Collaborative Laboratory at Weill Cornell Medicine and a Member of the Board of the Ludwig Institute for Cancer Research, were elected in April as Fellows of the Academy of the American Association for Cancer Research (AACR), class of 2023. The Academy honors scientists whose research has contributed significantly to our understanding of cancer biology or fueled innovation in cancer prevention and care. Out of George's many accomplishments, the Academy highlighted his "seminal contributions to characterizing the occurrence of spontaneous immune responses in ovarian tumors, for demonstrating that tumor infiltrating lymphocytes may function as strong predictors of enhanced ovarian cancer survival and for designing and implementing critical immunotherapy clinical trials." Similarly, in Jedd's case, the Academy selected from a sprawling body of notable science his "illustrious contributions to novel cancer immunotherapy strategies, such as demonstrating the benefits of checkpoint inhibitor therapy in melanoma and leading several pivotal clinical trials, including the phase I clinical trial for ipilimumab and the phase III clinical trial involving the combination of ipilimumab and nivolumab." Nominees to the AACR Academy are elected through an annual peer review process and ratified by the Executive and other committees.

Ludwig Lausanne's Johanna Joyce recognized by EACR as a leader in cancer research

Ludwig Lausanne's Johanna Joyce was named the recipient of this year's Pezcoller-Marina Larcher Fogazzaro-EACR Women in Cancer Research Award in recognition of her academic excellence, scientific leadership and contributions to our understanding of the tumor microenvironment, most notably the immune cell landscape of primary and metastatic brain tumors and their potential manipulation for therapy. Aside from highlighting this substantial body of high-impact discoveries, her advisory role at multiple research institutes and the widespread recognition she has received for her research, the selection committee noted that "Johanna is a committed mentor and advisor to many young researchers and a widely recognized advocate—in particular for women in cancer research." Johanna will attend the European Association for Cancer Research 2023 Congress titled 'Innovative Cancer Science ' in Torino, Italy, in June 2023 as an honored guest, where she will deliver the award lecture.

Johanna Joyce

Rachel Reinhardt

Rachel Reinhardt joins University of Arizona Health Sciences Office of Communications

We bid a fond farewell in late April to Rachel Reinhardt, senior vice president for communications at the Ludwig Institute for Cancer Research. Rachel has taken herself and her enviable talents across the country to serve as associate vice president of the University of Arizona Health Sciences Office of Communications. An alumnus of Yale University, Rachel joined Ludwig in 2011, arriving at the New York office from the Juvenile Diabetes Research Foundation. Before that, she was senior director of global communications at the International AIDS Vaccine Initiative, overseeing its scientific, advocacy and community-directed communications in sub-Saharan Africa, South Asia, Europe and the U.S. Rachel left a lasting mark at Ludwig, establishing the Ludwig Cancer Research brand, showcasing Ludwig's life-changing science—a tagline she came up with—and creating mechanisms, like the Scientific Insights webinars, to better connect the globally dispersed Ludwig Community. (Though, we note only in passing, totally unbiased experts consider Ludwig Link her greatest accomplishment in the last category.) In her new, prettier habitat—Tucson, no less!—Rachel will help publicize UArizona Health Sciences discoveries, connect scientists across its research hubs and support its medical outreach to underserved Native American and Hispanic communities. We'll all miss her, of course, but—having known her all its life—the Link's sure she's going to have a great time out there.

Ping-Chih Ho

Ping-Chih Ho promoted to full Member of Ludwig Lausanne

Ludwig Lausanne's Ping-Chih Ho was made a full Member of the Ludwig Institute for Cancer Research, Lausanne Branch, upon his promotion to tenured professor at the University of Lausanne in January. Ping-Chih has in recent years led a series of groundbreaking studies on the molecular crosstalk between tumor metabolism and the immune system, and his laboratory is a leader in the emerging field of immunometabolism. He and his team have discovered numerous mechanisms by which the metabolic microenvironment of tumors disables distinct elements of the anti-tumor immune response and identified several strategies to counter those defenses. Most notably, perhaps, Ping-Chih and his colleagues recently illuminated a new dimension of tumor evolution when they demonstrated that the immune system's surveillance of cancer can itself induce metabolic adaptations in the cells of early-stage tumors that simultaneously promote their growth and equip them to suppress lethal immune responses (see page 15). The study detailed the mechanism by which this "immunometabolic editing" of emergent tumors occurs in mouse models of the skin cancer melanoma and identified several potential drug targets to undermine the process for cancer therapy. Ping-Chih's creativity and productivity were most recently recognized with a Swiss Bridge Award and the Lloyd J. Old STAR award from the Cancer Research Institute.

Ludwig Scientific Advisor Christopher Walsh dies at 79

Christopher Walsh, who served from 2011 to 2016 as a scientific advisor to the Ludwig Institute for Cancer Research, died on January 10, 2023, from injuries sustained in a fall. An enzymologist of considerable renown, Chris was also famed for his research on antibiotics, especially his elucidation of the mechanisms of antibiotic resistance. He was among the founders of the discipline of chemical biology and the author of more than 800 scientific articles and 10 books, including a classic of his field, *Enzymatic Reaction Mechanisms*. Chris demonstrated his scientific acumen at an early age. Working with the legendary biologist E.O. Wilson and John Law as an undergraduate researcher at Harvard College, he identified the pheromone with which fire ants mark their scent trails, publishing his discovery in July, 1965, in *Nature*. He then earned a PhD at Rockefeller University and completed a postdoctoral fellowship at Brandeis University before joining MIT in 1972, where he rose to become chair of the Department of Chemistry. Chris was recruited to Harvard Medical School as the founding chair of the Department of Biological Chemistry and Molecular Pharmacology in 1987 and served from 1992-95 as president of Dana-Farber Cancer Institute before returning to full-time research. He was the Hamilton Kuhn Professor of Biological Chemistry and Molecular Pharmacology, Emeritus, at Harvard Medical School at the time of his death.

Hugh Robson MacDonald in 1984

Former Ludwig Lausanne Director Hugh Robson MacDonald dies at 76

Hugh Robson MacDonald, a former director of the Lausanne Branch of the Ludwig Institute for Cancer Research at Epalinges, died in March at the age of 76 due to complications from a fall. A native of Canada, Rob obtained his PhD in biophysics from the University of Toronto, after which he took a fellowship at the Swiss Institute for Experimental Cancer Research in Lausanne from 1972-75, studying T cell differentiation. After a stint at the University of Western Ontario in Canada, Rob returned to Switzerland in 1977 to join Ludwig Lausanne, becoming its director from 2007 to 2012. Rob made seminal contributions to the field of T cell development—in part through his pioneering use of flow cytometry—immune tolerance, T cell selection and the establishment of immune memory. His group also discovered and extensively characterized the invariant natural killer T cell. Writing in *Immunity*, his former Ludwig colleagues Anne Wilson, Isabel Ferrero, Daniel Speiser, Werner Held and Pedro Romero recalled how Rob was loved "for his bright intellect, phenomenal memory, great sense of humor, eclectic spirit, insatiable curiosity, and his trademark humility." Rob authored some 430 scientific articles that, as of 2018, had collected at least 34,000 citations. His life's work was recognized with the prestigious Swiss Cloëta Prize in 1989.

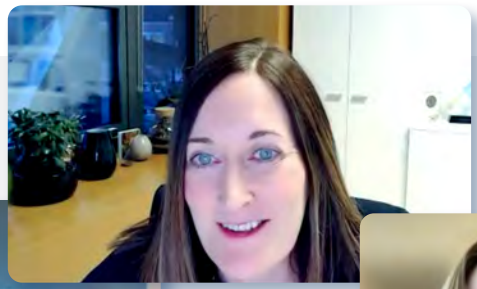
Herbert Oettgen

Herbert Oettgen, pioneering cancer immunologist, dies at 99

Herbert Oettgen, a renowned tumor immunologist and former chairman of the Protocol Review Committee at the New York office of the Ludwig Institute for Cancer Research, died on March 16, 2023. He was 99 years old. Born in Cologne, Herbert received his medical degree and completed residencies in Germany before arriving in New York City as a Fulbright scholar in 1958, conducting research in immunology and training in oncology at the Sloan-Kettering Institute. Moving to Kenya in 1960, Herbert participated in the development of a chemotherapeutic regimen for the treatment of Burkitt's lymphoma, which accounted for more than half the childhood cancer cases recorded in the country. In 1963, he returned to Sloan-Kettering, and was soon leading clinical trials on L-asparaginase, which would become a standard chemotherapy for childhood leukemia. Herbert, who worked closely with Ludwig's former Scientific Director and CEO Lloyd Old, became the founding head of the Division of Applied Immunology at Sloan-Kettering in 1970. He played a key role in developing Bacillus Calmette-Guérin and granulocyte-colony stimulating factor (G-CSF) as treatments for cancer patients. A prominent champion of cancer immunotherapy, Herbert ultimately joined the leadership of the Cancer Vaccine Collaborative launched by Lloyd Old. He retired in 2012, spending his last years caring for his wife and applying his formidable woodworking skills to making violins, which were played at his memorial service in May.

Joan Brugge

Nancy Davidson



Johanna Joyce

Crystal Mackall

Two panel discussions enriched the Ludwig community's ongoing conversation on women in science

Following up on the recent Ludwig publication, *Women in science: Perspectives from Ludwig leaders*, Ludwig Communications convened a pair of panel discussions with the accomplished scientists profiled in the report. Webcast as Ludwig Scientific Insights webinars, the discussions coincided with Women's History Month, with the first discussion held on March 8, which happened to be International Women's Day. That panel included Ludwig Harvard Co-director Joan Brugge, Ludwig Institute Board Member Nancy Davidson, Ludwig Lausanne's Johanna Joyce and Crystal Mackall of Ludwig Stanford. Discussions that day touched on everything from mentorship to the importance of nurturing family life and other activities outside the laboratory to the many options people with scientific training have in picking careers suited to their particular passions and talents.

The second panel discussion, webcast on March 22, was with Ludwig MIT's Sangeeta Bhatia, Ludwig Oxford Director Xin Lu, Ludwig Scientific Advisor Juanita Merchant and Ludwig Princeton Associate Director Eileen White. It too built on matters covered in the report, with the panelists touching on subjects ranging from their formative experiences as researchers to ways to support other women in science to their thoughts on how increasingly sophisticated AI applications are going to affect scientific research and future careers. If you missed these engaging discussions, you can catch both in the News section of the Ludwig Cancer Research website here. Happy viewing!

Xin Lu

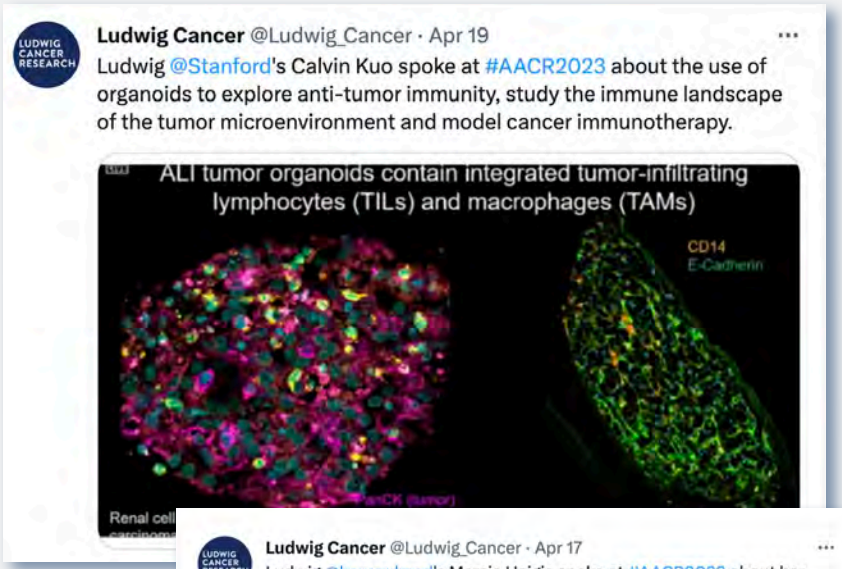
Sangeeta Bhatia

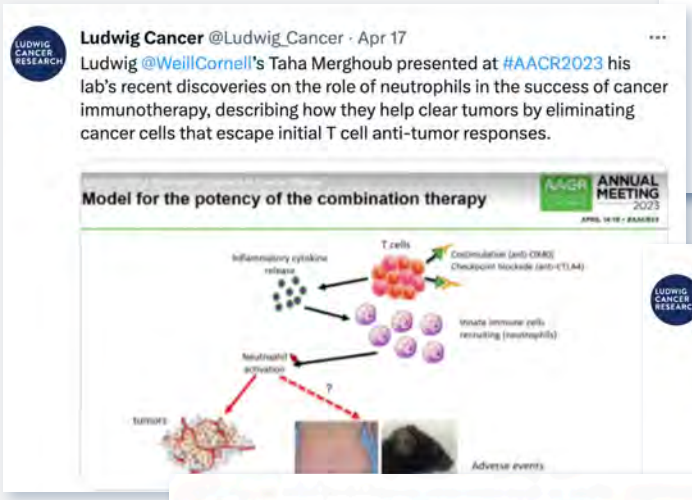
Eileen White

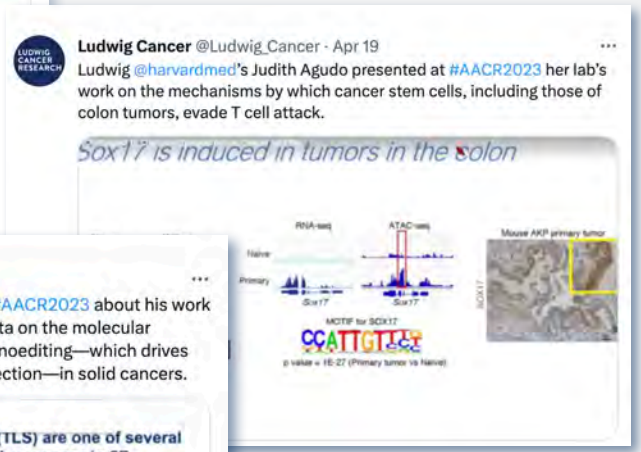
Juanita Merchant

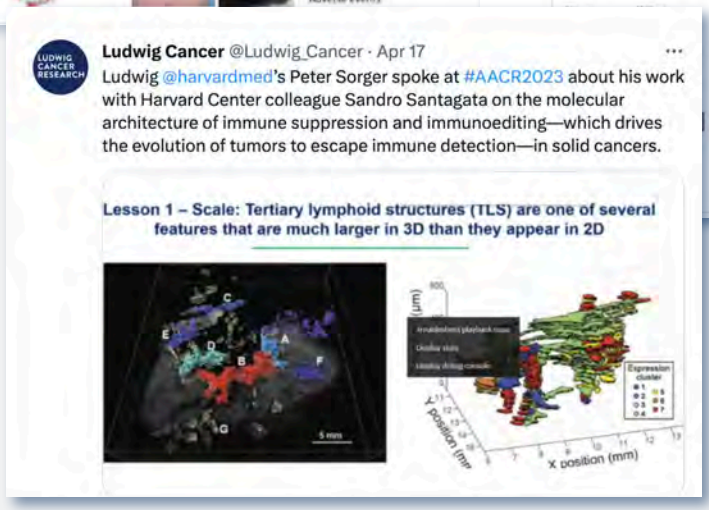
Ludwig at the AACR Annual Meeting 2023

Several investigators and trainees affiliated with Ludwig Cancer Research gave presentations, spoke at forums and "meet the expert" sessions or shared their research in poster sessions at the American Association for Cancer Research (AACR) Annual Meeting 2023, which was held online and in person in Orlando, Florida, from April 14 to April 19. Presentations covered everything from tumor immunology to cancer metabolism to drug resistance to "liquid biopsies" for the diagnosis and management of cancer—and a good deal more. Lists of Ludwig researcher presentations and posters can still be accessed via the press release on the News page of our website. Here's a sample of our tweets from the meeting.









AACR/Todd Buchanan

Six young scientists attend the AACR Annual Meeting with Ludwig support

Ludwig Cancer Research was proud to support six young scientists presenting their research at the conference this year through the AACR Scholar-in-Training Awards program, contributing to the cost of their travel and attendance at the conference. The SITA awards, as the AACR notes, "recognize outstanding young investigators presenting meritorious proffered papers" at the meeting. Award recipients were selected based on their CVs, personal statements and quality of research. Among the winners pictured above are, from left:

Jonathan Downie of Harvard Medical School/Massachusetts General Hospital, whose abstract was titled *The effect of aspirin on the transcriptional landscape of the colon*

Susana Castro Pando of the University of Texas MD Anderson Cancer Center, whose abstract was titled *IL-17/IL-17RA signaling in the pancreatic epithelium upregulates B7-H4 to promote tumorigenesis*

Pat Morin, deputy scientific director of the Ludwig Institute for Cancer Research

Robert Vonderheide, chair of the program committee for AACR 2023; Director of the Abramson Cancer Center, University of Pennsylvania

Carli Stewart of the Mayo Clinic, whose abstract was titled *IL-4 depletion leads to the improvement of CART cell therapy*

Jaime Schneider of Massachusetts General Hospital/Harvard Medical School, whose abstract was titled *GUK1 is a novel metabolic liability in oncogene-driven lung cancer*

Alvaro Curiel Garcia of Columbia University Irving Medical Center, whose abstract was titled *BMAL2 is a KRAS-dependent master regulator of hypoxic response in pancreatic ductal adenocarcinoma*

Not pictured: **Brendan Heiden** of Washington University in St. Louis, whose abstract was titled *Comprehensive validation of high-risk clinicopathologic features in early-stage, node-negative non-small cell lung cancer*

David Gfeller

Algorithms predicting T cell targets could aid development of personalized immunotherapies

A study led by Ludwig Lausanne's David Gfeller reported in *Cell Systems* in January the development and validation of machine learning algorithms (MixMHCpred and PRIME) that predict with high fidelity the antigens likely to be recognized by cytotoxic CD8+ T cells of the immune system. CD8+ T cells distinguish infected or malignant cells from normal ones by recognizing small protein fragments—or neoantigenic peptides—that are foreign or appear abnormal due to mutations that occur across the cancer genome. In humans, these neoantigens can be displayed on the surface of diseased cells by a class of proteins known as class I human leukocyte antigens (HLA-I). Yet not all suspect peptides in cells can be targeted by cytotoxic CD8+ T cells, as not all bind to HLA-I on cells, and even among those that bind to HLA-I, not all are seen by the receptors expressed on T cells. Predicting which peptides are likely to be presented by HLA-I and recognized by T cell receptors is valuable to the development of individualized cancer immunotherapies and interventions for infectious diseases. David and his colleagues applied their PRIME algorithm to identify several peptides associated with SARS-CoV-2—the virus that causes COVID-19—that are likely seen by CD8+ T cells and thus involved in immune responses to the infection. They then validated their predictions in laboratory studies. One such peptide from SARS-CoV-2 whose equivalents in other coronaviruses are also recognized by CD8+ T cells could be of value to the development of broader interventions to prevent or treat infection by these medically important viruses.

In another study, published in *Immunity* in April, David and his colleagues described a novel computational approach to better characterize how CD4+ T cells recognize neoantigens and predict which are most likely to elicit a functional response from the immune cells. These cells—which include the helper T cells that orchestrate adaptive immune responses—recognize antigens displayed on the surface of cells by MHC-II molecules (the equivalent of HLA-II in humans). The specificity of MHC-II molecules is not well understood, and identifying all peptides that are likely to elicit immune responses experimentally is both complex and costly. David's team analyzed more than a half million MHC-II ligands to get a better handle on MHC-II binding specificities. This revealed new structural mechanisms of MHC-II binding to peptides and allowed the researchers to improve predictions of the antigens targeted by CD4+ T cells using their MixMHC2pred software. Their software tool, the researchers reckon, could reduce by at least a factor of 100 the number of peptides that need to be experimentally verified as potential helper T cell targets in the design of personalized immunotherapies.

Improved predictions of antigen presentation and TCR recognition with MixMHCpred2.2 and PRIME2.0 reveal potent SARS-CoV-2 CD8+ T-cell epitopes | *Cell Systems*, 2023 January 4

Machine learning predictions of MHC-II specificities reveal alternative binding mode of class II epitopes | *Immunity*, 2023 April 5

Mechanisms by which mutant CALR drives myeloproliferative neoplasms

Frameshift mutations in the calreticulin (CALR) protein can cause slow-growing blood cancers known as myeloproliferative neoplasms (MPNs). Such mutations induce the stable binding of CALR to the thrombopoietin receptor (TpoR) within the same cell, which constitutively activates TpoR signaling, driving cell proliferation. In an April paper in *Nature Communications*, researchers led by Ludwig's Stefan Constantinescu reported the basis of the acquired specificity of CALR mutants for TpoR, how their binding triggers TpoR activation and how the complex might be targeted by drugs to treat CALR-mutant MPNs, which account for some 25% of essential thrombocythemia and myelofibrosis cases. Another study led by Stefan and published in the journal *Blood* in February reported that the mutant CALR binds and is secreted into the plasma in complex with soluble transferrin receptor 1 (sTFR1), which stabilizes it and extends its half-life. The complex, Stefan and his colleagues showed, can activate TpoR much like a rogue cytokine and can generate colonies in culture in the absence of thrombopoietin. They also found that TpoR-expressing cells with a CALR mutation are uniquely sensitive to the levels of circulating mutant CALR proteins seen in patients.

Stefan Constantinescu

Oncogenic CALR mutant C-terminus mediates dual binding to the thrombopoietin receptor triggering complex dimerization and activation | *Nature Communications*, 2023 April 5

Secreted mutant calreticulins as rogue cytokines in myeloproliferative neoplasms | *Blood*, 2023 February 23

Aaron Newman

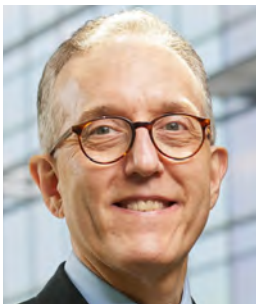
High-resolution alignment of single-cell and spatial transcriptomes with CytoSPACE | *Nature Biotechnology*, 2023 March 6

New method sharpens picture of how cellular location influences patterns of gene expression

With the development of increasingly sensitive molecular profiling technologies, researchers have in recent years sought to explore how the location of individual cells in tissues influences their behavior and global gene expression patterns, or transcriptomes. This capability is of importance to tumor biology since differences in local signaling networks can, by altering gene expression in individual cells, profoundly influence cellular heterogeneity, tumor progression and drug resistance. But existing technologies for this sort of "spatial transcriptomics" have relatively poor cellular resolution or are unable to detect the full scope of gene expression in individually resolved cells. That is, they either detect gene expression profiles in clusters of cells—typically ten or more in number—or fail to capture the full spectrum of genes expressed in individual cells. Researchers led by Ludwig Stanford's Aaron Newman reported in *Nature Biotechnology* in March a new method for spatial transcriptomics named CytoSPACE that solves both problems: it captures the full scope of gene expression while sharpening the spatial resolution of transcriptomes down to the level of individual cells. He and his colleagues show that CytoSPACE far outperforms previous methods in terms of its accuracy and single-cell resolution when employed for spatial transcriptomics in multiple types of tissue.

Mikaël Pittet

Taha Merghoub



Jedd Wolchok

Two independent studies place neutrophils front and center in successful immunotherapy

Two Ludwig studies published in a March issue of *Cell* independently reported that immune cells known as neutrophils, whose abundance in the tumor microenvironment has traditionally been associated with poor patient prognosis, can play an important role in the success of cancer immunotherapies. One study, co-led by Ludwig Lausanne's Mikaël Pittet identified a functional state assumed by neutrophils following immunotherapy—termed the *Sell*^{hi} state—in which they become formidable agents of anti-tumor immunity in mouse models of lung and colon cancer. The other, led by Ludwig Weill Cornell's Taha Merghoub and Jedd Wolchok, simultaneously discovered in a mouse model of melanoma that neutrophils are essential to the complete destruction of tumors during immunotherapies such as immune checkpoint blockade (ICB).

Mikaël and his colleagues showed in mice that neutrophil numbers surge in tumors that respond to immunotherapy. In responsive tumors in models of lung and colon cancer, it is neutrophils in the *Sell*^{hi} state that explode in number, and their expansion is essential to the success of immunotherapy. Examining the neutrophil activation, the researchers found that it depends on the production by dendritic cells of a factor (IL12) that activates killer T cells, which in turn produce a signaling protein named interferon-γ, further stimulating immune responses and—crucially—enabling the *Sell*^{hi} neutrophil response.

Taha, Jedd and their colleagues were using a mouse model for the study of antigenic heterogeneity in melanoma to explore the mechanisms by which an experimental therapy under development in their lab clears cancer cells that have evolved to escape T cell recognition. They found that tumors cleared by their immunotherapy in the mice were consistently infiltrated with activated neutrophils, and depleting neutrophils in the mice undermined the therapy. An analysis of tumor samples from melanoma patients treated with immune checkpoint blockade therapy revealed they too were teeming with neutrophils. Further, the researchers demonstrated that activated neutrophils contribute to tumor elimination in a model of colorectal cancer as well. This study too identified unique gene expression signatures and cell surface markers in tumor-targeting neutrophils. Notably, the function of activated neutrophils involved in clearing lingering cancer cell variants depends on the expression of an enzyme that drives production of nitric oxide and is associated with an enhanced cell killing capability in the immune cells. Taken together, the studies make a strong case for the selective engagement of neutrophils in combined immunotherapies for multiple cancers.

A neutrophil response linked to tumor control in immunotherapy | *Cell*, 2023 March 30

T cell immunotherapies engage neutrophils to eliminate tumor antigen escape variants | *Cell*, 2023 March 30

Mutations that could serve as prognostic markers of follicular lymphoma

Most follicular lymphomas harbor the BCL2 chromosomal translocation, but not everyone with this translocation develops the cancer, which diminishes its predictive value. To find the other mutations that cooperate with BCL2 translocation to initiate follicular lymphoma (FL), researchers led by Ludwig Stanford's Ash Alizadeh and Joseph Schroers-Martin conducted ultra-sensitive sequencing of prediagnostic blood, saliva and tissue samples from 48 people who ultimately developed the cancer among thousands enrolled in two early cancer detection studies. The researchers reported in *Cancer Discovery* in March that mutations to the lysine acetyltransferase (KAT) domain of CREB binding protein (CREBBP), a tumor suppressor, were the most common precursor lesions identified. They largely distinguished patients developing FL (14/48, 29%) from healthy adults with or without detected BCL2 rearrangements. Mutational variants of CREBBP could be detected nearly six years before FL diagnosis and were clonally selected in the cancers. The findings suggest that mutations of the CREBBP KAT domain are common in precancerous cells destined to become follicular lymphomas. Ash and his colleagues suggest such founder mutations might help identify people with BCL2 translocations likely to develop FL, and serve as a specific marker of common progenitor cells and residual disease following treatment.

Tracing founder mutations in circulating and tissue-resident follicular lymphoma precursors | *Cancer Discovery,* 2023 March 20

Ash Alizadeh

Joseph Schroers-Martin

Ping-Chih Ho

How immunometabolic editing drives immune evasion and tumor evolution

A study led by Ludwig Lausanne's Ping-Chih Ho discovered that the immune system's surveillance of cancer can itself induce metabolic adaptations in the cells of early-stage tumors that simultaneously promote their growth and equip them to suppress lethal immune responses. Published in a January issue of *Cell Metabolism*, the study detailed the precise mechanism by which this "immunometabolic editing" of emergent tumors occurs in mouse models of the skin cancer melanoma. Ping-Chih and his team found that three proteins—IFNγ, STAT3 and c-Myc—orchestrate the process. Secreted by immune cells, IFNγ blocks the growth of tumors. But its signaling via STAT1 also helps cancer cells adapt to evade T cell attack—a process known as immunoediting. The researchers found that IFNγ additionally activates a signaling pathway mediated by STAT3 that induces epigenetic changes and hyperactivates the master regulator of cell metabolism c-Myc, which is often aberrantly active in cancer. Several genes activated by c-Myc, they found, also compromise T cell function and infiltration into tumors. STAT1 and STAT3 signaling pathways, in fact, synergize to confer on emergent tumors the ability to evade immune responses, driving the immunometabolic editing that enables full-blown malignancy. The findings suggest STAT3 as well as 40 metabolic c-Myc-regulated genes involved in immunometabolic editing whose products could be prime targets for cancer therapy.

Immunoediting instructs tumor metabolic reprogramming to support immune evasion | *Cell Metabolism,* 2023 January 3

Geoffrey Shapiro

Polymerase θ inhibition activates the cGAS-STING pathway and cooperates with immune checkpoint blockade in models of BRCA-deficient cancer | *Nature Communications*, 2023 March 13

Experimental drug for BRCA-mutant cancers could boost efficacy of cancer immunotherapy

The polymerase θ inhibitor novobiocin, which induces a synthetic lethality in cancer cells, has recently been approved for clinical evaluation as a possible treatment for BRCA1/2 mutant cancers. Researchers led by Ludwig Harvard's Geoffrey Shapiro examined how the drug affects the tumor microenvironment in mouse models of BRCA1-deficient breast cancer and BRCA2-deficient pancreatic cancer. They reported in a March issue of *Nature Communications* that the drug causes chromosomal damage in cancer cells that results in the accumulation of micronuclei, which are free-floating bits of chromosomes. This activates the cGAS/STING pathway, an innate sensing system for cytoplasmic DNA that alerts the immune system to viral infection or DNA damage. cGAS/STING signaling led to the production of type 1 interferons, the activation of dendritic cells and an influx of killer T cells into the tumor microenvironment in mice. Novobiocin also boosted the expression of PD-L1 by cancer cells, an adaptive response to thwart T cell attack. The researchers showed that depleting killer T cells compromised the efficacy of novobiocin, while adding anti-PD1 immunotherapy enhanced anti-tumor effects in the mice. Geoff and his colleagues argue that their results provide a rationale for a clinical trial evaluating the combination of novobiocin and checkpoint blockade immunotherapy in patients with BRCA1/2 mutant cancers.

Chunxiao Song

Modular oxidation of cytosine modifications and their application in direct and quantitative sequencing of 5-hydroxy-methylcytosine | *Journal of the American Chemical Society*, 2023 March 24

CAPS+, a new procedure for epigenetic mapping, improves on a gold standard

Selective, efficient and controllable oxidation of cytosine modifications is valuable for epigenetic analysis. Researchers co-led by Ludwig Oxford's Chunxiao Song reported in a March paper in the *Journal of the American Chemical Society* a new method for that purpose. Their method integrates two new modular chemical oxidation reactions with borane reduction to develop a method they call CAPS+ for the direct and quantitative sequencing of hydroxymethylcytosine modifications on DNA. CAPS+ is an improvement on the chemical-assisted pyridine borane sequencing (CAPS) procedure for epigenetic mapping developed in Chunxiao's lab. Chunxiao and his colleagues showed that the oxidation reactions they employ cause very little damage to DNA, an asset in applying such methods to extremely small samples, such as those of liquid biopsies and single cells. The researchers tested the CAPS+ procedure by mapping 5hmC on DNA from mouse embryonic stem cells as well as tissues taken from normal human brain and glioblastoma tumors. Their studies confirmed previous findings that the 5hmC modification, which plays a role in multiple biological processes and is especially abundant in nervous system tissues, declines some seven-fold in abundance in glioblastoma tumors. Their analysis shows that CAPS+ generates fewer false positives and is more efficient than CAPS, which is already an extremely sensitive and efficient procedure for epigenetic sequencing.

Study shows cancer cells can be reprogrammed into living cancer vaccines

Researchers led by Ludwig Stanford's Ravi Majeti reported in a March publication in *Cancer Discovery* that they had reprogrammed cancer cells isolated from mouse models of four types of cancer into potent anti-tumor vaccines. Ravi's lab has previously shown that the cells of a type of leukemia can be reprogrammed to resemble macrophages, which are professional antigen presenting cells (APCs). The reprogrammed cancer cells retain genomic aberrations of their cancerous progenitors but are not malignant. Ravi and colleagues showed in this study that these "tumor-reprogrammed APCs" (or TR-APCs) can, like macrophages, present a broad spectrum of the cancer antigens encoded in their genomes to T cells, directing a concerted assault on cancerous cells. Use of the TR-APCs in a mouse model of leukemia potently activated helper T cells that orchestrate adaptive immune responses and killer T cells that destroy sick and cancerous cells, eradicating disease in the mice. Like living vaccines, the reprogrammed cells also instilled in the mice a memory of the cancer, prompting them to reject subsequent attempts to engraft the same cancer. The TR-APC vaccines also extended survival in mouse models of solid tumors, namely fibrosarcoma and breast and bone cancer. Ravi and his team demonstrated that TR-APCs derived from leukemia patients could activate T cells taken from those same patients in cell cultures, suggesting the approach might be ripe for translational development.

Ravi Majeti

Reprogramming cancer into antigen-presenting cells as a novel immunotherapy | *Cancer Discovery*, 2023 March 1

Reductive stress could be a chink in the armor of some lung cancers

To deal with oxidative stress, many cancers activate a regulator of gene expression, NRF2, by mutating the gene for its suppressor KEAP1. Although the molecular biology of such cancers has been extensively studied, less is known about those in which KEAP1 is not mutated. A study published in *Cell Metabolism* in February led by Ludwig Harvard's Liron Bar-Peled—and co-authored by Harvard Center colleagues Marcia Haigis and Aaron Hata—induced NRF2 activation by pharmacologically inactivating KEAP1 in a panel of more than 50 non-small cell lung cancer cell lines. The researchers found, surprisingly, that about 13% of the cell lines failed to survive when KEAP1 was inhibited, and that their viability could be restored by the ablation of NRF2. Examining this phenomenon, they discovered that NRF2 induces reductive stress in these cells by driving the production of ALDH3A1—an enzyme that consumes NAD+—causing an accumulation of the electron carrier NADH. Liron and his colleagues examined whether the buildup of electrons, or NADH-reductive stress, in these cells might be exploited to target cancers in which NRF2 is activated. They showed that such cancers are specifically vulnerable to inhibition of a core metabolic enzyme known as Complex 1 when their KEAP1 protein is inactivated pharmacologically or by genetic manipulation. Complex 1 inhibition boosts reductive stress by compromising NADH oxidation in these cancer cells, suggesting that reductive stress is a metabolic vulnerability of NRF2-activated cancers.

Liron Bar-Peled

NRF2 activation induces NADH-reductive stress, providing a metabolic vulnerability in lung cancer | *Cell Metabolism*, 2023 February 24

Ping-Chih Ho

CD40 signal rewires fatty acid and glutamine metabolism for stimulating macrophage anti-tumorigenic functions | *Nature Immunology*, 2023 February 23

How a candidate immunotherapy pushes macrophages into an anti-tumor state

Tumor-associated macrophages can enter a state in which they support tumor growth (the M2 phenotype) or, alternatively, one in which they attack cancer cells (M1), and can be prompted to switch states by a variety of factors. A team co-led by Ludwig Lausanne's Ping-Chih Ho examined how one such factor now being tested in clinical trials as a cancer therapy—the activation of the cell-surface signaling protein CD40—pushes macrophages into an M1 state. Other mechanisms that drive M1 polarization disrupt a central series of energy-harvesting reactions known as the TCA cycle and drive aerobic glycolysis, in which energy is extracted from glucose in the cytoplasm even in the abundance of oxygen.

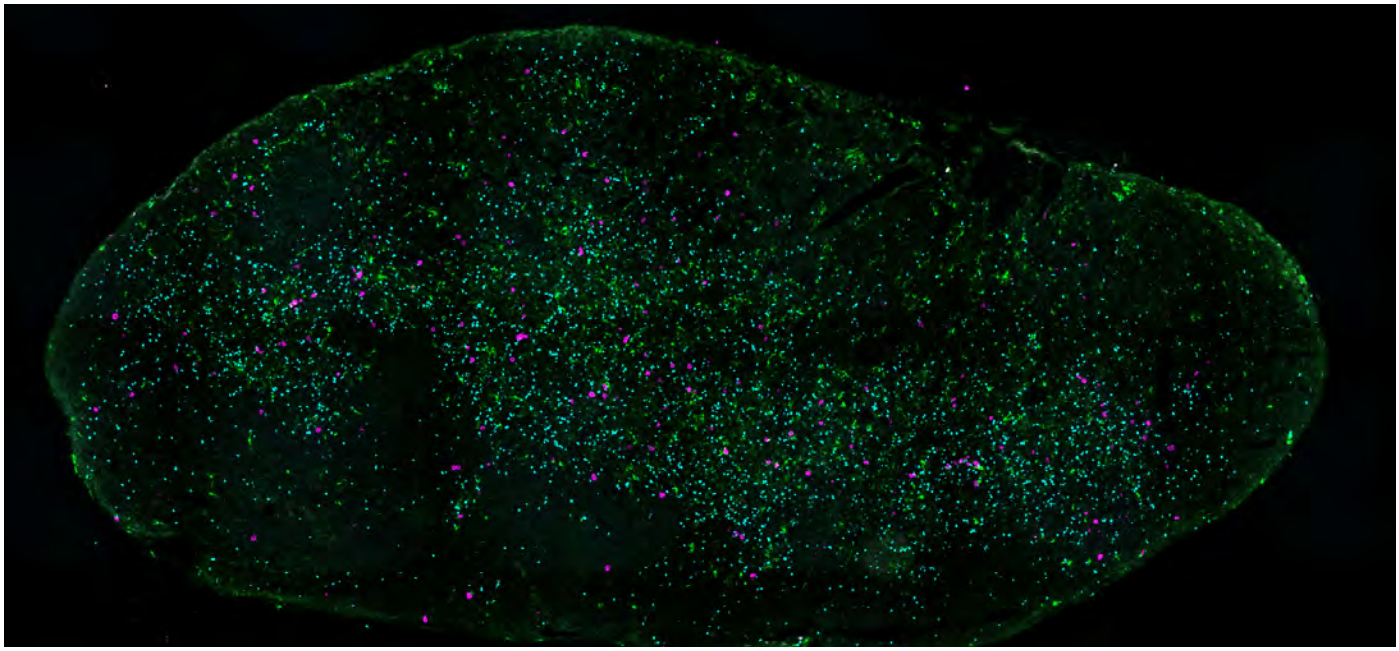
Ping-Chih and his colleagues reported in a February publication in *Nature Immunology* that CD40 activation does not disrupt the TCA cycle. Its signals instead switch on the metabolism of the amino acid glutamine and the oxidation of fats, which normally push macrophages into an M2 state. Under CD40 stimulation, however, the byproducts of those processes are used by the macrophage to epigenetically reprogram its genome, activating genes involved in anti-tumor responses. Ping-Chih and his colleagues argue their results support the use of drugs to precondition the metabolic state of tumor-associated macrophages prior to the use of CD40-activating therapies.

Xin Lu

Regulation of immunological tolerance by the p53-inhibitor iASPP | *Cell Death & Disease*, 2023 February 6

iASPP deficiency supports immune evasion in mouse models of cancer

Recent research has suggested that the tumor suppressor p53 mediates interactions between dying cells and the immune system, but the mechanisms by which this occurs were unclear. Researchers co-led by Ludwig Oxford Director Xin Lu suspected that iASPP, a regulator of p53, may be involved in that mediation and demonstrated in a February publication in *Cell Death & Disease* that the protein indeed regulates immune tolerance in response to cell death. This is of relevance to cancer progression because one way tumors can evade immune clearance is by promoting immunologically silent—or tolerogenic—responses to the detritus of their constituent cancer cells, which should normally elicit inflammatory immune responses. Xin and her colleagues showed that in models of lung and pancreatic cancer, deletion of the iASPP gene promoted tumorigenesis and the immune response to these tumors exhibited hallmarks of immunosuppression, such as the presence of activated regulatory T cells and exhausted CD8+ T cells. They found that iASPP-deficient tumor cells and tumor-infiltrating immune cells expressed elevated levels of PD-1H, a recently identified transcriptional target of p53 that promotes a tolerogenic immune response. iASPP deficiency thus seems to favor an immunosuppressive microenvironment in lung and pancreatic tumors, which in turn promotes tumor progression. The findings should open opportunities for the development of novel therapies against cancer and autoimmune disease.



A lung tumor-draining mediastinal lymph node, where dendritic cells fail to activate CD8+ T cells, compromising anti-tumor immune responses.

Interferon-γ drives T cell dysfunction and compromises immunotherapy in lung cancer

Stefani Spranger

A study led by Ludwig MIT's Stefani Spranger explored how factors unique to the tumor-draining mediastinal lymph nodes (mLNs) compromise CD8+ T cell responses to lung cancer. Stefani's lab had previously identified a gene expression signature in tumor-infiltrating T cells that is associated with an inability to target cancer cells. In the recent study, her team sought to understand the origins of that dysfunction. She and her colleagues reported in *Immunity* in February that the dysfunction originates in the mLNs, where tumor-targeting killer T cells should ordinarily be activated by antigen-presenting dendritic cells. Their studies revealed that Type 1 conventional dendritic cells (DC1s) fail to appropriately activate tumor-targeting killer T cells because they are directly suppressed by regulatory T cells (Tregs) in specific niches within the mLNs. The Tregs, the researchers found, are pushed by elevated levels of a factor known as interferon-γ (IFNγ) into a state in which they resemble T helper-1 (Th1) cells. IFNγ is produced in response to commensal bacteria that live in the lung. The researchers showed that the presence of Th1-like Tregs correlates to poor responses to checkpoint blockade therapy in patients. They also showed that in mouse models of lung cancer IFNγ-blockade reprogrammed Tregs out of the Th1-like state and restored CD8+ T cell-targeting of lung tumors.

Tissue-specific abundance of interferon-gamma drives regulatory T cells to restrain DC1-mediated priming of cytotoxic T cells against lung cancer | *Immunity*, 2023 February 2

Metabolomic probe uncovers energy misappropriation in primary tumors

A study led by Ludwig Princeton Director Joshua Rabinowitz and Caroline Bartman, a postdoc in his laboratory, measured the rates of glycolysis and the TCA cycle—cellular energy production processes—in normal mouse tissues, five primary tumor types and metastases using a quantitative method that involves labeling molecules with heavy isotopes and tracing their metabolic fates. The researchers used these data to calculate total rates of ATP production, a measure of how much usable energy was being produced in each tissue type. While 95% of ATP in healthy tissues comes from the TCA cycle, the absolute rates of energy production from that source or glycolysis have never quite been determined for malignant tissues. Josh, Caroline and their colleagues reported in a *Nature* paper in February that primary tumors, long assumed to make and consume large quantities of energy, actually convert nutrients to usable energy at relatively low rates. This was not true, however, for lung metastases of breast cancer. Malignant tissues, the researchers propose, support their abnormal cell proliferation in part by neglecting energy investments in previously normal tissue functions. In the pancreatic tumor model, for example, the cancer cells scavenged the energy they needed for proliferation by slowing down protein synthesis, a major energy-hog in the healthy tissue. The findings have implications for therapies that seek to starve tumors to stall their growth.

Slow TCA flux and ATP production in primary solid tumours but not metastases | *Nature*, 2023 February 1

Joshua Rabinowitz

Caroline Bartman

Johanna Joyce

Ángel Álvarez-Prado

Charting the immunogenomic landscapes of brain metastases

A study led by Ludwig Lausanne's Johanna Joyce and Ángel Álvarez-Prado combined a panoply of molecular profiling and imaging techniques to explore whether immune cells in breast and lung cancer brain metastases (BrM) behave differently depending on the mutations present in the host tumor's cells. Brain metastases are the most common type of brain tumors and are associated with poor outcomes, with lung and breast cancer patients surviving just 7 to 9 months, respectively, following diagnosis. Clinicians are increasingly using genetic analysis to personalize treatment of these tumors and while immunotherapeutic approaches to their treatment—though long ineffective—are now beginning to show promise, little was known about how their genomic landscapes affect their heterogeneous immune microenvironments. Johanna, Ángel and their colleagues reported in a January issue of *Cell Reports Medicine* that TP53 mutant lung-BrMs are more infiltrated with immune cells and have more activated killer T cells as well as more immunosuppressive tumor-associated macrophages than those lacking such mutations. In breast-BrMs, hypermutation—seen in 44% of the metastases the researchers analyzed—was associated with greater numbers of infiltrating killer T cells into the tumor and a generally more inflamed immune microenvironment. These findings, the authors suggest, should contribute to future efforts to devise immunotherapies that are tailored to better fit the immunogenomic landscapes of brain metastases.

Immunogenomic analysis of human brain metastases reveals diverse immune landscapes across genetically distinct tumors | *Cell Reports Medicine*, 2023 January 17

Two studies reveal distinct ways a generic blood pressure drug can enhance cancer therapy

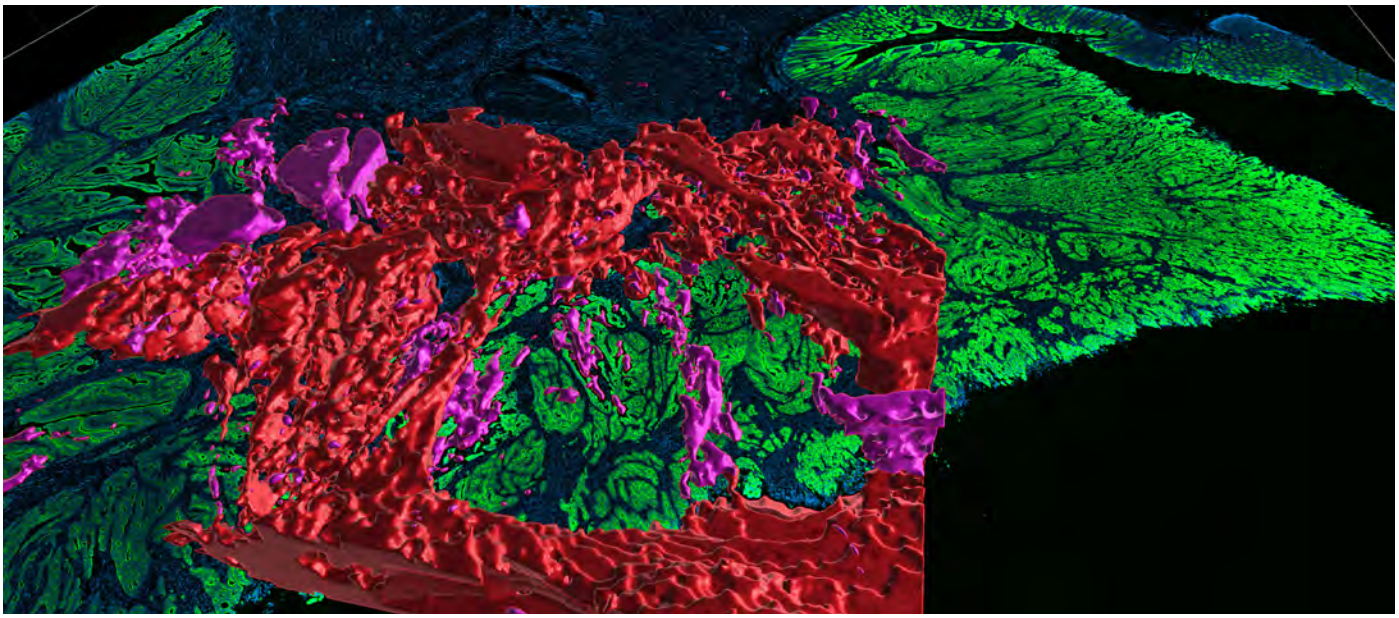
Researchers led by Ludwig Harvard's Rakesh Jain reported in a study published in *PNAS* in February why anti-PD1 immune checkpoint blockade (ICB) induces edema in some glioblastoma (GBM) patients and in mouse models of the cancer. Using single-cell RNA sequencing, intravital imaging and CD8+ T cell blocking studies in mice to study the phenomenon, Rakesh and his colleagues determined that the edema is caused by an inflammatory response following anti-programmed death 1 (PD1) therapy that disrupts the blood–tumor barrier. They showed in mouse models that the edema, which is normally managed with high doses of corticosteroids, can be better treated with the antihypertensive drug losartan. The generic angiotensin receptor blocker, they found, reduces expression of membrane-type matrix metalloproteinases that are upregulated in tumor endothelial cells during ICB therapy and that disrupt the blood-brain barrier. It also increased the blood perfusion of tumors, reprogrammed the tumor microenvironment and improved survival in two out of three preclinical models, curing 20% of the mice. That cure rate doubled when the treatment was complemented with standard of care therapy. The researchers also describe an immunologic signature and key biomarkers that predict which mice were likely to respond well to the combination therapy. The results, they argue, support evaluation of the drug combination in patients. It certainly helps that both drugs are already available and widely used in the clinic.

In another paper published a week later in *Clinical Cancer Research* a team of researchers led by Rakesh explored the mechanisms underlying losartan's ability to enhance therapy for locally advanced pancreatic cancer. The study analyzed surgical samples obtained from patients enrolled in a phase 2 trial of a therapy combining pre-surgical treatment with losartan and a cocktail of chemotherapies (FOLFIRINOX) plus radiotherapy in which 61% of patients responded so well that their primary tumors could be completely excised. The researchers found that the combination therapy altered expression of genes associated with immunosuppression, cancer invasion, the health of blood vessels and antigen presentation by the immune system's dendritic cells. In patients who responded best to the combined therapy, the tumor microenvironment had fewer suppressive regulatory T cells and immunosuppressive cancer cells, and larger numbers of CD8+ T cells, suggesting the drug combination counters immunosuppression in the tumor microenvironment. Taken together, these findings suggest losartan enhances the effects of chemoradiotherapy by alleviating the immunosuppression of the tumor microenvironment.

Rakesh Jain

Losartan controls immune checkpoint blocker-induced edema and improves survival in glioblastoma mouse models | *PNAS*, 2023 February 1

Addition of losartan to FOLFIRINOX and chemoradiation reduces immunosuppression-associated genes, Tregs, and FOXP3+ cancer cells in locally advanced pancreatic cancer | *Clinical Cancer Research* | 2023 February 7



Three-dimensional reconstructions of a colorectal tumor reveal that pools of mucin (red and pink), which appear to be isolated structures in 2D images, are often connected to one another and to the colon lumen. Image by Clarence Yapp, Sorger lab.

3D maps of colorectal tumors uncover novel structures, molecular gradients and cellular interactions

Peter Sorger

Sandro Santagata

A Ludwig Harvard team led by Peter Sorger and Sandro Santagata applied highly multiplexed tissue imaging and 3D reconstruction methods along with spatial statistics and machine learning to identify cell types and states underlying morphological features of known diagnostic and prognostic significance in colorectal cancer. Their large scale 2D and 3D maps, reported in a January issue of *Cell*, revealed key immune interactions at the invasive boundary of tumors, interconnections between histological structures and morphological and molecular gradients of the sort typically seen in developing tissues. Lymphoid structures that were previously thought to be isolated and pools of mucin, for example, were each revealed in 3D reconstructions to be highly interconnected. Peter, Sandro and their colleagues also showed that regions within tumors vary in their degree of malignancy and their invasive properties. Gradients in histological features and the expression of oncogenes and epigenetic regulators of gene expression reflect transitions between more and less invasive regions. On the immunological front, the Ludwig Harvard team found that it is mainly other immune cells—namely, myeloid cells—and not just cancer cells that suppress anti-tumor T cell responses by engaging the PD-1 immune checkpoint. The study is part of Ludwig's Tumor Atlas project and the National Cancer Institute's Human Tumor Atlas Network (HTAN). To learn more and access the dataset, visit the Harvard Tissue Atlas.

Multiplexed 3D atlas of state transitions and immune interaction in colorectal cancer | *Cell*, 2023 January 19

Cell free DNA analysis reveals determinants of failure of CAR-T cell therapy for LBCL

Researchers led by Ludwig Stanford's Ash Alizadeh and Brian Sworder profiled over 700 longitudinal specimens from two independent cohorts of relapsed/refractory large B cell lymphoma (r/rLBCL) patients treated with CAR-T cells targeting CD-19 to identify determinants of resistance to this therapy. Relapse is seen in roughly half of such patients. The researchers developed a method called STEP to simultaneously profile tumor DNA, CAR-T cell effectors and receptor rearrangements of ordinary T cells in the blood and integrated these profiles to assess treatment failure. They also constructed a multivariate model incorporating these features and used it to predict outcomes. Ash, Brian and colleagues reported in a January paper in *Cancer Cell* that alterations in multiple classes of genes are associated with treatment resistance, including those affecting B cell identity (PAX5 and IRF8), immune checkpoints (CD274) and the microenvironment (TMEM30A). Somatic tumor alterations influence the efficacy of anti-CD19 CAR-T therapy at multiple levels, including CAR-T cell expansion and persistence and conditions of the tumor microenvironment. They found that the genotype and phenotype of tumors influence the expansion of CAR-T cells, which can in turn shape those characteristics of tumors. The researchers suggest their findings could inform the development of improved and personalized CAR-T cell therapies for LBCL.

Ash Alizadeh

Brian Sworder

Douglas Hanahan

Mélanie Tichet

Determinants of resistance to engineered T cell therapies targeting CD19 in large B cell lymphomas | *Cancer Cell*, 2023 January 9

Targeted cytokine is highly effective against pancreatic cancer in mice

A study co-led by Ludwig Lausanne's Douglas Hanahan and Mélanie Tichet evaluated the effects of an engineered immunocytokine, PD1-IL2v, in a mouse model of pancreatic neuroendocrine cancer that is resistant to immunotherapy. A Roche product composed of an anti-PD1 targeting moiety fused to an immuno-stimulatory IL-2 cytokine variant (IL2v), PD1-IL2v selectively delivers the cytokine—which can otherwise be quite toxic—to PD1-expressing T cells in the tumor microenvironment. The researchers reported in an *Immunity* paper in January that PD1-IL2v induced high-endothelial venules (vascular structures that promote T cell trafficking) and substantial infiltration of stem-like, tumor-reactive killer T cells into tumors. This resulted in tumor regression and enhanced survival. Combining the immunocytokine with an anti-PD-L1 checkpoint blockade antibody sustained these therapeutic effects. Douglas, Mélanie and colleagues found that the combination reprogrammed both immunosuppressive tumor-associated macrophages and the tumor vasculature to assume antigen-presenting and pro-inflammatory phenotypes and enhanced diversity of the T cell receptor repertoire. The authors suggested these results predict benefits of the combination therapy in clinical trials. Notably, Roche has launched such a trial (NCT04303858).

Bispecific PD1-IL2v and anti-PD-L1 break tumor immunity resistance by enhancing stem-like tumor-reactive CD8+ T cells and reprogramming macrophages | *Immunity*, 2023 January 10

Kornelia Polyak

JAK–STAT signaling in inflammatory breast cancer enables chemotherapy-resistant cell states | *Cancer Research*, 2023 January 3

Potential combination therapy for an aggressive type of breast cancer

A team led by Ludwig Harvard's Kornelia Polyak, and including Harvard Center researchers Alex Toker and Franziska Michor, examined mechanisms of drug resistance in inflammatory breast cancer (IBC), an aggressive form of the disease that is highly metastatic and often resistant to therapy. They reported in a January paper in *Cancer Research* that a cell type they previously identified, defined as CD44+CD24-, is the most common cell type in IBC tumors, and that most such cells are phospho-STAT3+. Kornelia and her colleagues had also previously shown that such CD44+CD24-STAT3+ cells are dependent on JAK2/STAT3 signaling. In the recent study, they showed that combining inhibition of JAK2/STAT3 with paclitaxel treatment suppressed the growth of IBC xenografted tumors in mice more than either agent could alone. Studies using a cell line that mimics clinical drug resistance revealed that STAT3 regulates genes related to inflammation and epithelial-to-mesenchymal transition (EMT)—an initiating step in the metastatic cascade—as well as an enzyme that acts on the signaling molecule cAMP. Metabolic analyses stemming from this observation identified elevated cAMP signaling and CREB as potential therapeutic targets in IBC. The researchers showed that paclitaxel plus JAK2/STAT3 inhibition suppresses the emergence of the chemotherapy-resistant subpopulation of IBC cells, an observation that further supports the use of such drug combinations in the treatment of IBC.

Chi Van Dang

Lactate-dependent chaperone-mediated autophagy induces oscillatory HIF-1α activity promoting proliferation of hypoxic cells | *Cell Systems*, 2022 December 2

Solving a paradox of hypoxic metabolism reveals driver of cancer cell proliferation

A study co-led by Ludwig Institute Scientific Director Chi Van Dang used a reporter of HIF-1α activity in hypoxic cancer cells to track the temporal dynamics of the oxygen sensing protein in individual cells and explore a paradox of its effects on cancer cell metabolism. When oxygen is in low supply, HIF-1α —which controls a complex program of gene expression—can drive glycolysis, in which energy is extracted from sugar in a manner that produces an acid named lactate. Glycolysis and the normal processes of energy extraction from sugar—mitochondrial respiration—tend to be mutually exclusive. Yet HIF-1α seems able to activate both processes in cancer cells, an inconsistency in its effects that was not quite understood. Chi and his colleagues reported in a *Cell Systems* paper in December that HIF-1α-driven gene expression oscillates in a small subpopulation of hypoxic cancer cells. The oscillations, they show, are driven by lactate and associated in these few cells with the intermittent use of mitochondrial respiration; most hypoxic cells, however, continue to rely on glycolysis. They also show that lactate can serve as a nutrient and messenger to convey information about cell density in hypoxic subregions of the tumor. The oscillatory HIF-1α activity in the few cells pushes them out of the cell cycle arrest induced by hypoxia, inhibits the expression of tumor suppressor genes and promotes the expression of oncogenes that drive cancer cell proliferation.

MICHAL BASSANI-STERNBERG

Explorer of the immunopeptidome

Michal's pioneering work on the recognition of cancer antigens is being applied to the design of novel immunotherapies

Michal Bassani-Sternberg's group at Ludwig Lausanne, the immunopeptidomics unit, has become a key participant in the Branch's ambitious efforts to develop, optimize and streamline the delivery of personalized immunotherapies to cancer patients. Michal is uniquely qualified for the role. As a postdoc in Germany, she 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. Her high-throughput technologies are today integral to the development and clinical evaluation of individualized cancer vaccines and adoptive T cell therapies at the Branch and the Center of Experimental Therapeutics at the University of Lausanne. On the basic research front, her laboratory is making invaluable contributions to our understanding of the rules that govern antigen presentation and recognition. Ludwig Link recently caught up with Michal for an interview about her life, work and more. Here is an excerpt of our conversation.

"We are looking for the very few peptides that are unique to a cancer and are not presented anywhere else in the body. Once we identify those peptides, we can develop immunotherapies to target them and induce anti-cancer immune responses in patients—for example, by vaccination."

Where were you born and raised?

I was born and raised in Israel, in Haifa, a city in the north, close to the sea. And I grew up there in a very normal family. I have an older sister, younger brother, and they're all still living there in Israel. My father worked as a chemist in the paint industry and my mother worked as a secretary in the hospital.

Where and what did you study from college onward?

I attended the Technion, which is the Technical University of Israel, and studied biology there as an undergraduate and got my master's degree and PhD there as well. My master's was in plant physiology and it was in a very nice laboratory. I learned a lot of scientific techniques and became independent as a researcher early on, but the topic was not very interesting for me. After two years of that I said, okay, I need to change direction. So I transitioned into cancer immunology, in which I got my PhD, getting involved in immunopeptidomics in the early years of the field. I just moved from one floor to the other in our faculty building, but I had to learn so many new things about cancer and immunology and become familiar with a completely different technology, which was mass spectrometry. This is when I started to explore HLA-bound peptides and measure them with mass spec.

What are HLA-bound peptides?

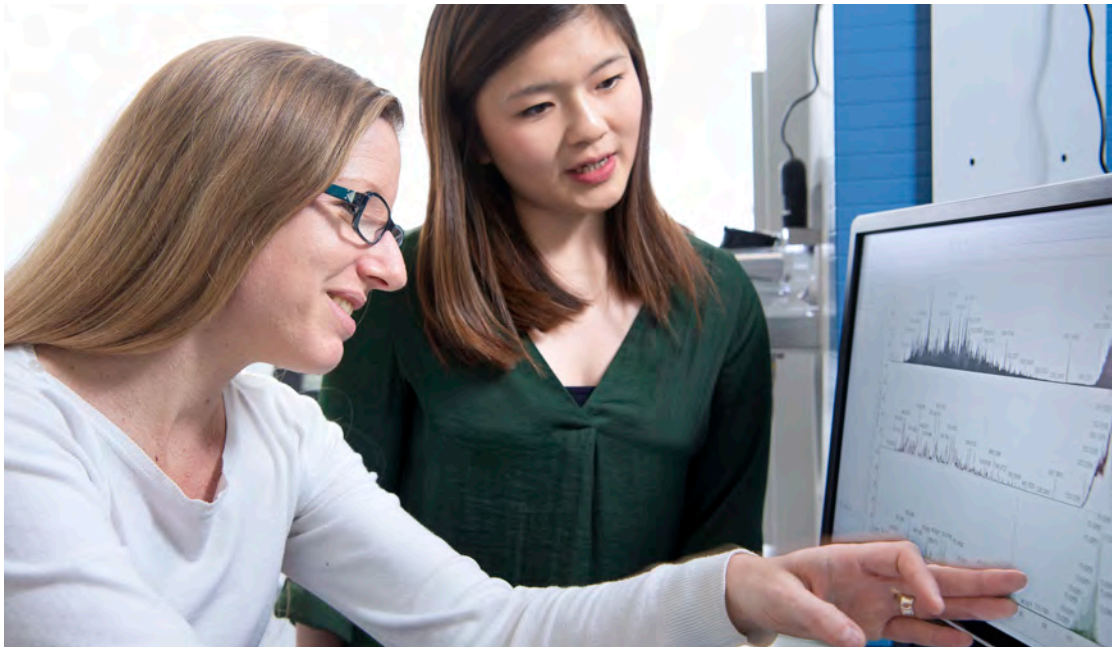
Normally, cells present cellular proteins to the immune system as short peptides bound to human leukocyte antigen (HLA) molecules on the cell surface. When cells become diseased by cancerous transformation or infection, they present altered, new or non-self peptides that can be recognized by T cells and induce a T cell response. The one line of work that really picked up and developed eventually into my thesis was after we realized that we could identify HLA-bound peptides from plasma samples of cancer patients. This was a completely new discovery and application, and it was really at the very early stages when the technology was not yet ready for this crazy idea. But we saw it as a proof of concept.

What did you do next?

During my PhD I got married, I had my two kids and then we moved the family to Munich, Germany, where my husband was going to do his postdoc. I also did my postdoc there, in a department that remains a world leader in mass spectrometry and proteomics. I continued work in immunopeptidomics, but now in the best place in the world to do mass spec and proteomics. It was very inspiring to be at the first line of innovation in this domain and to apply it to my scientific questions. People were working on many applications of proteomics, while I was the only one doing immunopeptidomics there at that time.

Could you tell us what immunopeptidomics is?

Immunopeptidomics is a technique by



Aside from its practical applications, Michal's work is elucidating important principles of antigen presentation and recognition, which are early steps in the initiation of adaptive immune responses.

which we isolate, identify and analyze large numbers of HLA-bound peptides. Most of the peptides that are presented derive from normal proteins, but we are looking for the very few that are specific to disease, in our case, cancer. We are looking for the very few peptides that are unique to a cancer and are not presented anywhere else in the body. Once we identify those peptides, we can develop immunotherapies to target them and induce anti-cancer immune responses in patients—for example, by vaccination.

So you must coordinate with T cell biologists in this work.

Exactly. We ask, what are the relevant targets in any given tumor? It's a personalized approach. And we are trying to collect as much information as we can. We do genome sequencing, RNA sequencing and peptidomics—analyzing the full repertoire of peptides that are presented by cancer cells—and we try to find all the abnormalities within the cells that could lead to the presentation of cancer specific targets. We aggregate all this information and come up with the most likely immunogenic antigens, and then we work with our colleagues here in Lausanne, for example, Alex Harari's group, who are experts on T cell recognition assays. They test whether the antigenic peptides we propose are recognized by autologous T cells of the patient and which of them are immunogenic. We work very, very closely with them.

What scientific challenges does your work address?

Well, there are different challenges. There's the more conceptual challenge—to understand which classes of antigens we should target or explore further. These could be mutations or different flavors of mutations; they could be abnormally translated regions in our genome, or

"We are trying to understand the rules of what makes an antigen immunogenic. And we are trying to understand this now more and more in the context of the immune microenvironment of the tumor, and how that affects the immune responses provoked by antigens."

posttranslational modifications made to proteins. For every source of antigens, we need to collect different data or develop different computational tools for their robust identification. So we analyze these patient by patient and then try to go as deeply as possible and characterize as many different types of antigens of interest as we can. And yeah, it's growing quickly. Every few months we come up with a different source of antigens we need to explore, and we have to adapt our pipelines and algorithms for their analysis. We are trying to understand the rules of what makes an antigen immunogenic. And we are trying to understand this now more and more in the context of the immune microenvironment of the tumor, and how that affects the immune responses provoked by antigens.

Then there are practical challenges related to the tissue samples that we receive, which are sometimes very, very small. We are developing new methods to analyze them by mass spec. And, of course, there are challenges related to the fact that every tumor comes from a different individual. The patient may have received prior treatment that could change the tumor and there is not a lot of information about how different treatments affect the antigenic landscape in cancer. This is a topic we are very interested in exploring.

How is this work contributing to medicine?

We have put together in recent years a very complex, high throughput proteogenomics pipeline that is quite exceptional, which can identify in a patient's tumor the various antigens that are presented and are likely to elicit immune responses. We have applied it to research in the lab, but also to ongoing clinical trials in Lausanne. So we can now explore the antigenic landscape at a level where we can propose relevant targets for the development of immunotherapies. And I think immunopeptidomics is a field still in its early days, in terms of its potential impact on immunotherapy. But the technology is incredibly better today than it was even a few years ago, in terms of its sensitivity and speed and robustness as well as the computational tools that support it.

What trials are utilizing this technology right now?

We're involved in cancer vaccine trials and adoptive T cell therapy trials. For cancer vaccine trials, we propose the most relevant mutations in tumors for use in personalized cancer vaccines. The antigens these mutant genes encode are produced and loaded on dendritic cells from the patient, which are then offered as a vaccine to the patient with the expectation that they will induce immune responses against the cancer cells that present these antigens. We are also involved

in the NeoTIL trial, which involves adoptive T cell transfer. In this trial, we again propose the neoantigenic peptides that might be most relevant and they are produced and used in the selective, *in vitro* expansion of neoantigen-specific T cells obtained from patients. Those T cells are then infused into the patient from which they were isolated as a personalized immunotherapy.

What cancers are you trying to treat using these techniques?

We have vaccine trials in pancreatic and lung cancers and we just started one for ovarian cancer. The NeoTIL program is in various solid tumors.

How has the environment in Lausanne enabled you to do things that maybe you couldn't have done otherwise?

It was George's vision, when he recruited me here in 2015, to establish the immunopeptidomics lab and develop our proteogenomics approaches within the oncology department. So this is quite a translational environment. To bring such technology into a clinical environment is quite unique. We are being challenged to do things very robustly, to do things in a very, very careful way, but also to innovate and challenge ourselves with scientific questions that are linked to the burning questions and needs of the clinic.

Congratulations on the Swiss Bridge Award. Could you tell us about the project you proposed?

The project supported by the Swiss Bridge Award will bridge two distinct domains—infectious disease and cancer. A lot is known about cancers that are induced by viral infection, like HPV for example. There is already information about which targets could be immunogenic and we realized that we have now an opportunity in the lab to explore with our pipelines what are really the peptides that are naturally processed and presented in tumors or model cell lines of these cancers. We will also try to understand how different drugs change the peptidome, applying high throughput methods and screening approaches to try to see how different drugs and their combinations alter the expression and presentation of viral peptides with the hope of developing immunotherapies that target those antigens.

You said your husband is also a researcher, what does he study?

He studied astrophysics, but in recent years he has transitioned more into data science in oncology and other life sciences.

What global issues concern you the most?

What has really kind of shocked me is climate change. We feel its effects every day here in Switzerland. You would assume the kind of changes people anticipate will take tens of years to happen. But no, we feel them every day, and every year it's getting worse and worse.

What are your main interests and hobbies?

We hike a lot on the mountains here in the summer and in winter we ski with the family and friends. I would say the normal hobbies in Switzerland. Also, I have been doing hot yoga for the past two years or so, and I really enjoy it. It's 90 minutes in a very hot room, so you sweat a lot. Funnily, it's exactly like the weather on a normal summer day in my hometown in Israel. It's very demanding on the body but it's quite excellent. It's good for the soul and for the body as well. It's a good meditation while you do a very intense sport.

Whether you're a young scientist or a senior researcher, which area of cancer research do you believe holds the greatest promise for someone now starting up a career in science?

SUDIPTA GHOSH
Ludwig Oxford

Cancer is very complex and chaotic in terms of its genetic signature. However, we still maintain a "hope among the chaos" for a better treatment by new discoveries and new therapeutic strategies. Among many encouraging areas, newly discovered RNA modifications and their interplay with RNA splicing possibly hold a promise for future therapeutic opportunities in cancer.

Cancer research is a fascinating field, with many questions still to be answered. Being a PhD student, I would say that it is not that much about the specific area in cancer research, but more about the people you are going to work with. Scientists with different backgrounds could complement each other, joining forces and expertise. A multidisciplinary environment holds great promise for new breakthroughs.

AMANDA WICKI
Ludwig Oxford

RICHARD WHITE
Ludwig Oxford

Our understanding of the proteome lags far behind that of DNA and RNA alterations in cancer. In part, this is due to a lack of scalable technologies like we have for nucleic acids. A concerted effort to map the cancer proteome (at both single-cell and spatial levels) is a major challenge for the next 10 years.

Obesity and cancer. There is an obesity epidemic in most modern nations, which is most prominent in the USA. There are many cancers that are caused by obesity and the mechanisms are unknown. Thus we are facing a cancer epidemic likely related to changes in the food supply, nutrition and lifestyle, with no clear way to address it, that will impact millions of people.

EILEEN WHITE
Ludwig Princeton

Research on the tumor macroenvironment, to understand how the tumor influences and is modulated by other tissues and cell states in the human body, holds significant potential for improved cancer prevention and treatment. For example, why do tumors initiate and progress more efficiently in obese, sedentary or aged people? Do tumors metastasize more efficiently to insulin resistant liver? How does the tumor induce adipose and muscle wasting in cancer cachexia?

KAY MACLEOD
Ludwig Chicago

RALPH WEICHSELBAUM
Ludwig Chicago

If I were just starting out I would develop expertise in bioinformatic analysis. It is becoming increasingly clear to me that these types of analyses are key to the understanding of biological data, developing new hypotheses and integration with clinical outcomes, among many uses.

The area of research that holds the greatest promise is the one that consumes you—you can't stop thinking about it because you believe it will eventually decrease suffering and death from cancer.

BERT VOGELSTEIN
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