September 2022

Ludwig Link

LUDWIG CANCER RESEARCH

LIFE-CHANGING SCIENCE

Welcome

Rachel Reinhardt
Senior Vice President for Communications

Welcome to the fall issue of Ludwig Link! As you will likely notice, our newsletter is slightly changed in its design and layout. The refresh was prompted to some degree by a change in its distribution: long a periodical shared only within the extended Ludwig community, this newsletter is now posted on our website and available to anyone anywhere interested in cancer research. We couldn't think of a good reason why all of the contributions Ludwig researchers make to the field should not be shared with the rest of the world.

What hasn't changed, of course, is the high quality of research reported in these pages. You'll read in this issue, for example, about a new strategy for improving the efficacy of natural killer cell therapies, how defects in autophagy might drive a chronic intestinal disorder that increases the risk of GI cancers and how a computational model exploring the emergence of mutational hotspots in the cancer genome reveals a tradeoff made by evolving tumor cells that could be exploited for preventive therapy in some people prone to developing cancer. And that's just a small sample of the notable discoveries reported by Ludwig researchers in recent months.

You'll find, in addition, the usual reports of honors and awards issued to our researchers and a Q&A introducing the Ludwig Institute's new Deputy Scientific Director Pat Morin to our community. We also have a feature on a transnational collaboration on liquid biopsies—launched by Ludwig a dozen years ago—for managing the treatment of colon cancer that more than proved its worth in a major clinical trial. Finally, for our "Ask a scientist" feature, we asked our community to tell us what they think is the most significant change they made during the pandemic that has made them more effective at work. Read on to find out what they said.

We hope you enjoy this fall issue.

Sincerely,

Rachel

On the cover

This acrylic painting on canvas by Sudipta Ghosh, a postdoctoral fellow at Ludwig Oxford, portrays an invading cancer cell reshaping its environment. The chaos in the cancer cell is complex and daunting, but technological and scientific advances give us reason for hope that the cellular dysfunctions of cancer, and the disease itself, can be cured or tamed.

STORY ON PAGE 18

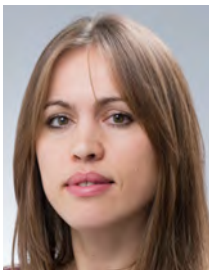
In this issue

Wild Type was "the band that we started when I was in the Kinzler-Vogelstein lab. We played at various scientific meetings and various events to raise money for research."

Q&A with Pat Morin
page 20

FEATURED RESEARCH

Researchers examined whether circulating tumor DNA could consistently predict disease recurrence after surgery for stage II colon cancer. They also sought to settle a debate over the necessity of post-operative chemotherapy in such patients. **Page 8**



"Prioritizing what is important and not just what is urgent is one of the most important lessons for optimal time management."

Denarda Dangaj

page 25

Yang Shi elected member of European Molecular Biology Organization

Yang Shi

Ludwig Oxford's Yang Shi was elected member of the European Molecular Biology Organization (EMBO). Yang was recognized by EMBO for his significant contributions to epigenetic research, which explores how chemical modifications made to chromatin influence the expression of the human genome. In 2004, Yang and his colleagues identified and characterized an enzyme, LSD1, that erases methyl marks from histones. Their discovery upended a 40-year-old dogma that considered such modifications irreversible, altering long standing models of genomic regulation.

His lab went on to identify numerous other histone demethylases and described their roles in an array of biological processes. More recently, his group has discovered that LSD-1 inhibition can spark anti-tumor immune responses even against immunologically "cold" tumors, as well as sustain T cell reinvogoration and promote durable tumor response to checkpoint blockade. Yang and his colleagues have also recently identified several new enzymes that methylate RNA, creating new opportunities to investigate RNA modifications in gene expression regulation and cancer.

Karen Oegema

Arshad Desai

Karen Oegema and Arshad Desai elected to American Academy of Arts and Sciences

Ludwig San Diego's Karen Oegema and Arshad Desai have been elected members of the American Academy of Arts and Sciences in recognition of their contributions to cell and developmental biology. Karen has made landmark discoveries on the role centrosomes play in the proliferation of healthy and malignant cells, which often have multiple copies of the organelle. Her lab has also developed powerful systems to study the functional genetics of embryogenesis using the roundworm *C.elegans* as a model system. Arshad has made major contributions to our understanding of how dividing cells parcel out equal numbers of chromosomes to their daughter cells. His lab has identified molecular checkpoints and other critical mechanisms that enforce fidelity in that process and are essential to ensuring genome stability during cell division. In collaboration with Karen's lab, Arshad's team has also been exploring the therapeutic potential of centrosome-targeting for cancer therapy.

Eileen White is a team leader for Cancer Grand Challenges award focused on cachexia

Ludwig Princeton Associate Director Eileen White was selected as a team leader for a $25 million Cancer Grand Challenges award to tackle cachexia, a debilitating wasting condition associated with advanced cancers. Though it contributes heavily to cancer mortality, cachexia is poorly understood and essentially untreatable. Eileen will, in partnership with Weill Cornell Medicine's Marcus DaSilva Goncalves and Tobias Janowitz of the Cold Spring Harbor Laboratory, lead a network of scientists based in the U.S. and U.K. known as the CANCAN (Cancer Cachexia Action Network) team. One of four teams selected in the latest round of Cancer Grand Challenges awards, the CANCAN team will explore the biological mechanisms of cachexia and identify potential therapies for its treatment. It will seek to build a "virtual cancer institute" dedicated to tackling the disorder, drawing together clinicians, advocates and scientists with expertise in cancer, metabolism, immunology and other biomedical fields at 14 institutions in the U.S. and U.K. Another team that includes Ludwig Stanford investigators Howard Chang and Michelle Monje and is led by Stanford professor Paul Mischel will investigate the generation of extrachromosomal DNA and its contributions to cancer evolution and drug resistance. You can learn more about the Cancer Grand Challenges and the award-winning teams in this Cancer Research UK article and this news release issued by the National Cancer Institute.

Flynn Larsen

Eileen White in her lab.

Mathematical biologist Helen Byrne joins Ludwig Oxford

Helen Byrne, a professor of mathematical biology at the University of Oxford whose research program operates at the interface of mathematical modeling and cancer biology, joined the Ludwig Oxford Branch in May as a senior group leader. Helen has been prominent in establishing productive collaborations between these fields in Oxford, leading projects on tumor growth, interactions with the microenvironment and the immune system, vascular networks and responses to therapy. Her group will extend its mechanistic models and advanced data analysis methods at Ludwig Oxford and develop innovative ways to combine these with complex, multiscale biomedical datasets. This approach should eventually establish a rational basis to support clinical decision-making, particularly for personalized cancer medicine. In recognition of her outstanding scientific achievements and record of active leadership in mentoring scientific careers, Helen was awarded the 2019 Leah Edelstein-Keshet Prize from the Society for Mathematical Biology, of which she became a Fellow in 2020. Helen retains her position at the Mathematical Institute in Oxford, and we expect her joint appointment will inspire new multidisciplinary partnerships and support the Oxford Branch's vision of improving the understanding of the molecular basis of cancer and its heterogeneous responses to therapy.

Helen Byrne

Ludwig Institute names Pat Morin deputy scientific director

In July, Pat Morin joined the Ludwig Institute for Cancer Research as deputy scientific director. Pat comes to us from the University of Pennsylvania, where he was executive director for strategic alliances at the Abramson Cancer Center and an adjunct professor at the Perelman School of Medicine. After obtaining his PhD at Boston University in 1995, Pat started his research career as a postdoc studying the Wnt/β-catenin pathway in colorectal cancer in the laboratory led by Bert Vogelstein and Ken Kinzler at what is now the Ludwig Center at Johns Hopkins University. He continued as an adjunct assistant professor at Johns Hopkins and joined the National Institute on Aging of the U.S. National Institutes of Health as a tenure-track investigator studying ovarian cancer, rising to the position of senior investigator at the institute. From 2012 to 2016, he served as the senior director of scientific review and grants administration at the American Association for Cancer Research, where he helped lead the Stand Up to Cancer initiative, before joining the Abramson Cancer Center in 2016. Pat will work with Scientific Director Chi Van Dang and CEO Ed McDermott to direct the Institute's research activities, select and review scientific staff and help develop and direct strategic initiatives and research collaborations. We extend a warm welcome to Pat on behalf of the larger Ludwig community.

Read our Q&A with Pat Morin, page 20

Pat Morin

A pioneering, 12-year long transnational Ludwig collaboration on liquid biopsies yields a major win

Circulating Tumor DNA Analysis Guiding Adjuvant Therapy in Stage II Colon Cancer
New England Journal of Medicine, 2022
June 4 Epub

A study conducted by Ludwig Johns Hopkins researchers, their colleagues at Johns Hopkins University and Ludwig alumni at the Walter and Eliza Hall Institute of Medical Research (WEHI), in Melbourne, examined whether circulating tumor (ct)DNA could consistently predict disease recurrence after surgery for stage II colon cancer. It also sought to settle a debate over the necessity of post-operative chemotherapy in such patients. The researchers—including Ludwig Johns Hopkins MD/PhD student and co-lead author Joshua Cohen, PI Nickolas Papadopoulos and Co-directors Bert Vogelstein and Ken Kinzler, and Jeanne Tie and Peter Gibbs at WEHI—reported June 4 at the Annual Meeting of the American Society of Clinical Oncology and in a publication in the *New England Journal of Medicine (NEJM)* that ctDNA can predict risk of recurrence in these patients and so identify those most likely to benefit from chemotherapy.

ctDNA is shed by the dying cells of tumors into the bloodstream and other fluids and has lately become a focus of scientists racing to develop minimally invasive diagnostics for the early detection and treatment of cancer. The current study has its roots in a 2010 Ludwig conference on colon cancer at Johns Hopkins University, where Gibbs—then a veteran scientist at the Ludwig Branch in Melbourne—first heard Bert describe his team's efforts to detect ctDNA in patients who had undergone surgery to remove liver metastases of colon cancer. Those with the highest levels of the biomarker, they found, were most likely to experience recurrence.

Joshua Cohen

Nickolas Papadopoulous

Bert was interested in confirming these findings. Peter, a clinician with expertise in treating colon cancer and conducting clinical research, had access to the many patients required for a larger, confirmatory study, as well as infrastructure in place to collect and store their tumor and blood samples for analysis, combined with comprehensive data on their treatment and outcomes. Collaboration seemed a no-brainer to both researchers.

By 2011, the pair, working with Jeanne Tie and others at their Ludwig labs and host institutions, had enrolled the first patients in that study and were soon expanding the scope of their project, drawing support from external funders, including the Conrad N. Hilton Foundation and Australian government agencies. In 2015, their efforts became the core clinical project of a five-year, $10 million initiative launched by Hilton Foundation and Ludwig Cancer Research for the prevention and early detection of colon cancer. The initiative has continued apace, with additional

Bert Vogelstein

Ken Kinzler

Jeanne Tie

Peter Gibbs

support from multiple public and private funding institutions adding to that of the Ludwig-Hilton initiative.

The study reported in *NEJM* established that ctDNA detection is a sufficiently sensitive method for the detection of micrometastases, or malignant growths far from the original tumor that can seed cancer recurrence. This confirms and considerably extends the findings the research teams reported in 2016 in *Science Translational Medicine* showing that ctDNA assays can detect residual disease after surgery for stage II colon cancer—and can be used to guide therapy.

For the *NEJM* study, the researchers enrolled 455 patients with stage II colon cancer and randomized them after surgery, with 153 receiving standard care—including monitoring for recurrence—and 302 receiving blood tests for ctDNA within seven weeks after surgery. Patients in the latter group received fluoropyrimidine or oxaliplatin-based chemotherapy only if ctDNA was detected in their blood. Just 15.3% of patients in the ctDNA-guided group received chemotherapy, versus 27.9% in the standard management group. Rates of two and three-year survival without recurrence were similar in the two groups. As notably, the researchers also demonstrated that treating only those who tested positive for ctDNA reduced chemotherapy use overall, sparing those who did not need the treatment its associated toxicities without compromising recurrence-free survival. This will doubtless come as a relief to many patients diagnosed with stage II colon cancer.

> **The researchers found that ctDNA can predict risk of recurrence and identify those most likely to benefit from chemotherapy.**


Chunxiao Song

A new technique adds to the expanding suite of TAPS capabilities

In a new paper published in *Nucleic Acids Research*, a team led by Ludwig Oxford's Chunxiao Song reported a method for whole-genome long-read sequencing using TAPS (for Tet-assisted pyridine borane sequencing), the method his and Benjamin Schuster-Böckler's groups published in 2019 for the detection of cytosine methylation and hydroxymethylation. Unlike other DNA methylation sequencing methods, TAPS does not rely on the harsh chemical bisulphite, which degrades a lot of the DNA sample. This has allowed Song's group to adapt the method for applications such as liquid biopsies. Long-read sequencing, meanwhile, permits the mapping of repetitive and complex genomic regions, which can reveal new information about the genome. Chunxiao and his team show that whole-genome long-read TAPS uncovered many sites of methylation that were not present in data from short-read TAPS. The method also enabled the detection of allele-specific methylation in imprinting genes.

Whole-genome long-read TAPS deciphers DNA methylation patterns at base resolution using PacBio SMRT sequencing technology | *Nucleic Acids Research*, 2022 July 18

Ludwig MIT study explains why hypoxic cancer cells must import their lipids to multiply

Proliferating cells need a standard electron-accepting molecule known as NAD+ for the oxygen-driven production of large biomolecules, which are essential building blocks of cells. A June publication in *Nature Metabolism* co-led by Ludwig MIT's Matthew Vander Heiden quantitatively demonstrated that when NAD+ is in short supply—as it is in low-oxygen conditions that are common in tumors—reactions that generate the precursors of lipids shut down, creating a supply bottleneck that snuffs out lipid biosynthesis. The shortage of oxygen does not seem to tax energy supplies in the cancer cells tested, however, as giving them more energy-generating molecules did not affect their proliferation. As much as 30% of the oxygen used by cancer cells, it turns out, can go to lipid biosynthesis. Matthew and his colleagues found that the access to a metabolic byproduct known as acetate can relieve dependency on NAD+ for lipid synthesis because it allows cells to bypass the NAD+-dependent steps of lipid production. They also showed that across tumor types, the elevated expression of genes involved in responses to oxygen starvation is accompanied by low expression of genes essential to lipid production. The study provides a mechanistic explanation for why cancer cells depend on imported lipids for growth during oxygen starvation and suggests that drugs that interfere with lipid import may be useful for cancer therapy.


Matthew Vander Heiden

Cancer cells depend on environmental lipids for proliferation when electron acceptors are limited
Nature Metabolism, 2022 June 23

Ashley Cook

Nicholas Wyhs

Kenneth Kinzler

Shibin Zhou

A new tool to screen drugs that target loss of tumor suppressor genes

In a May publication in *iScience*, researchers led by Ludwig Johns Hopkins's Ashley Cook, Nicolas Wyhs, Kenneth Kinzler and Shibin Zhou described their creation of a panel of isogenic—or genetically identical—cell lines designed to enable the large-scale screening of drugs that target cancers driven by the loss of function of tumor suppressor genes. The majority of cancer-driving mutations in tumors disable tumor suppressors, and many tumors are driven exclusively by such loss of function mutations. Yet only one drug approved by the U.S. Food and Drug Administration targets such a driver mutation. This species of cancer drivers can be targeted by disrupting new and unique biochemical dependencies that are generated in cancer cells by the loss of a critical gene function. The 100 cell lines developed by the Ludwig Johns Hopkins team permit the discovery of such dependencies because they are genetically identical to a corresponding line except in the functionality of one of 19 tumor suppressor genes. The cells encode DNA barcodes to enable multiplex screening, and the researchers developed an assay for high throughput drug screening using their cells. They validated their system by confirming that the Wee1 inhibitor MK-1775 is a selective growth inhibitor of cancer cells characterized by loss of function of the tumor suppressor TP53.

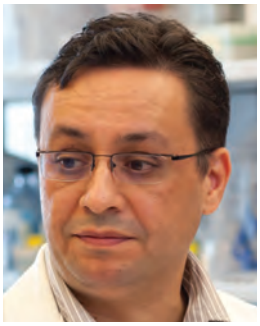
The majority of cancer-driving mutations in tumors disable tumor suppressors, and many tumors are driven exclusively by such loss of function mutations.

An isogenic cell line panel for sequence-based screening of targeted anticancer drugs | *iScience*, 2022 June 17

A potential therapeutic vaccine strategy for a subset of MPNs

The cells of a subset of myeloproliferative neoplasms (MPNs), slow-growing blood cancers, have frameshift mutations in their calreticulin (CALR) gene that are associated with the disease. The tail end of this gene's mutated protein product ($CALR^{MUT}$) should be an ideal neoantigen. Yet T cells directed against the $CALR^{MUT}$ fragment are rarely found in MPN patients. To figure out why, Ludwig MSK's Taha Merghoub and his colleagues examined the class I MHC genes—which present antigens to T cells—of patients with this kind of MPN. When T cells see these antigens, they are activated and kill the cells that express them. Taha and his colleagues found that these patients tend to lack MHC-I proteins that bind strongly to $CALR^{MUT}$ neoantigens, which may explain why their mutant cells aren't eliminated by the immune system before they can cause MPNs. The researchers generated mutant versions of $CALR^{MUT}$ peptides that bind efficiently to the MHC-I proteins found in these patients but retain the sequences that are recognized by T cell receptors. These so-called "heteroclitic" peptides, they reported in a November paper in *Science Translational Medicine*, elicited a cross-reactive CD8+ T cell response to $CALR^{MUT}$ in human blood samples, while the original peptides did not. The researchers verified these findings in mice as well. Taha and his team suggest on the basis of these studies that heteroclitic peptide-based cancer vaccines might represent a promising therapeutic approach for $CALR^{MUT}$ MPN patients.

Calreticulin mutant myeloproliferative neoplasms induce MHC-I skewing, which can be overcome by an optimized peptide cancer vaccine | *Science Translational Medicine*, 2022 June 15 Epub



Taha Merghoub

Anoek Zomer

Davide Croci

Johanna Joyce

A window into the changing tumor microenvironment

Researchers led by Ludwig Lausanne's Anoek Zomer, Davide Croci and Johanna Joyce described in a June publication in *iScience* their development of a multimodal imaging platform to explore, over extended periods, the changing interactions between various components of the brain tumor microenvironment (TME) in mice. To capture these cellular and molecular interactions as they occur in the brain TME, Johanna and her colleagues developed an imaging platform that combines magnetic resonance imaging with high-resolution two-photon intravital microscopy, which enables observation of the behavior of individual cells in living animals. They integrated into this platform powerful data science tools for single cell imaging analysis and applied this multifaceted system to track the changing behavior of hundreds of thousands of individual cells in the same animal models over several weeks, capturing an extremely detailed portrait of the changing TME during cancer progression. They also interrogated how therapies targeting tumor-associated macrophages affect the migration of these cells and analyzed how the dynamics and behavior of such macrophages differs between genetically distinct gliomas. The platform will be very beneficial for the study of brain tumor progression and the development of new therapeutic strategies for glioblastoma.

Multimodal imaging of the dynamic brain tumor microenvironment during glioblastoma progression and in response to treatment | *iScience* 2022 June 9

Rakesh Jain

Multiphoton Phosphorescence Quenching Microscopy Reveals Kinetics of Tumor Oxygenation during Antiangiogenesis and Angiotensin Signaling Inhibition | *Clinical Cancer Research*, 2022 May 18 Epub

Imaging method charts oxygenation of cells and tumors and suggests a cancer therapy

A team led by Ludwig Harvard's Rakesh Jain developed a new method of microscopy to image oxygen levels within cells and tissues in relation to blood vessels and applied it to observe the heterogeneity of oxygenation within tumors. The blood vessels that feed tumors tend to be very leaky, resulting in poor perfusion and oxygenation that contributes to resistance to both chemo and immunotherapies. Rakesh has proposed and extensively vetted the hypothesis that normalizing tumor blood vessels could help overcome the drug resistance of many solid tumors. He and his colleagues have shown that the blood supply to tumors is compromised by their leakiness and by compressive forces associated with the tumor mass. The former, they've shown, can be reversed with drugs that inhibit signaling through the vascular endothelial growth factor receptor (VEGFR)—which promotes angiogenesis—while the latter can be overcome with angiotensin system inhibitors. Both approaches have been or are currently being evaluated in clinical trials. In a May paper in *Clinical Cancer Research*, Rakesh and his colleagues reported that both the antiangiogenic blockade of VEGFR2 (using antibody DC101) and an angiotensin-receptor blocker (losartan) improve oxygenation in mouse tumor models, but to varying degrees depending on tumor type and dosage. Their findings also suggest that combining the two therapies is likely to achieve the best results.

A new type of cancer vaccine that could be broadly effective against cancers

Researchers led by Ludwig Harvard's Kai Wucherpfennig reported in a May *Nature* paper the design and preclinical evaluation in mice and primates of a new type of cancer vaccine that could be broadly effective against cancers. Vaccines elicit immune reactions to antigens, so they often need to be personalized to be effective against cancer due to the enormous variability of both the randomly mutated peptides presented as "neoantigens" and the major histocompatibility complex molecules that present them to T cells. Complicating the task further, cancers can evolve mechanisms to thwart peptide presentation. The new vaccine targets the MICA and MICB (MICA/B) stress proteins expressed on the surface of human cancer cells in response to DNA damage. MICA/B binding can activate natural killer (NK) and T cells through the NKG2D receptor, but cancer cells snip the telltale proteins off their surface to evade such responses. Antibodies elicited by the new vaccine interfere with that snipping, leaving MICA/B intact to stimulate NK and T cell activation. Kai and his colleagues showed that their vaccine is safe and effective, elicits an enhanced assault on cancer by both NK and T cells, and works even against tumors with a resistance mutation in the MHC class I antigen presentation pathway. Notably, it prevents the resurgence of metastases in animal models after the surgical removal of aggressive primary tumors.

Kai Wucherpfennig

A vaccine targeting resistant tumours by dual T cell plus NK cell attack | *Nature*, 2022 May 25 Epub

How intestinal cancer stem cells evade anti-angiogenic therapy

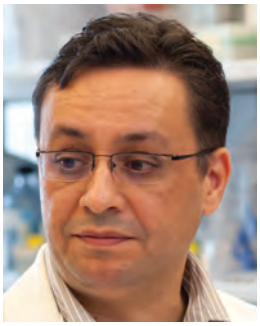
A team led by Ludwig Lausanne's Tatiana Petrova, Jeremiah Bernier-Latmani and alum Christoph Cisarovsky identified a novel mechanism by which stem cells in intestinal tumors generate new blood vessels and evade anti-angiogenic therapy. Vascular endothelial growth factor A (VEGFA) is an important supporter of both healthy and malignant angiogenesis. But anti-VEGFA therapies have had mixed success against many cancers, including colorectal cancer (CRC). In CRC tumors that resist anti-VEGF-A therapy, intestinal stem cells—or epithelial progenitor cells—tend to signal strongly through the WNT pathway compared to those that are susceptible to the therapy. Tatiana and her team explored in mouse models how such WNT^{high} cells, both normal and cancerous, maintain their vascular support. They reported in a May paper in *Nature Cardiovascular Research* that both types of progenitor cells are nestled in intestinal niches that are rich in oxygen and surrounded by VEGFA-independent vessels. The proliferation of cancerous epithelial progenitor cells induces the production of a small protein named apelin, which triggers the migration of endothelial cells from distant veins towards the progenitor cell niche, where they fill in existing blood vessels. This supports the coordinated growth of the epithelial progenitor cells and that of the blood vessel network independent of VEGFA. Loss of apelin inhibits progenitor cell proliferation, niche oxygenation and tumor growth, suggesting a new approach to treating CRC tumors that resist VEGFA blockade.

Apelin-driven endothelial cell migration sustains intestinal progenitor cells and tumor growth | *Nature Cardiovascular Research*, 2022 May 16 Epub

Tatiana Petrova

Jeremiah Bernier-Latmani

Christoph Cisarovsky



Taha Merghoub

Model of emergence of mutational hotspots suggests a therapy

Though the mutations in cancer cells are believed to occur randomly, certain regions of genes essential to cancer growth are more frequently mutated than others in established tumors. A study co-led by Ludwig MSK's Taha Merghoub modeled the evolution of mutational hotspots and experimentally verified the interplay between the selection of such mutations and their tendency to invite an immune attack on tumor cells. Its findings, reported in a May issue of *Nature*, reveal a tradeoff that guides tumor evolution and might potentially be exploited for both cancer prevention and therapy. Modeling the emergence of mutational hotspots in the tumor suppressor TP53, Taha and his colleagues showed that mutations that alter protein function in ways that benefit tumors cannot simultaneously evade immune surveillance. Their model anticipated overall survival of patients in several cohorts in The Cancer Genome Atlas and correctly estimated the age of cancer onset for people diagnosed with Li-Fraumeni syndrome, who tend to develop cancer due to inherited mutations in their TP53 genes. Their study also suggests that, early in their growth, tumors favor the proliferative boost offered by certain driver gene mutations over the risk they pose of immune detection. Because such targetable neoantigens are more often displayed in precancerous tissues, their immunotherapeutic targeting might help prevent the emergence of malignancy, especially in people who have an inherited proclivity for cancer.

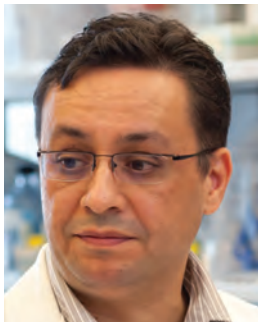
Fundamental immune–oncogenicity trade-offs define driver mutation fitness | *Nature*, 2022 May 11 Epub

Exploring the biology of 'double positive' T cells

The molecular markers CD4 and CD8—defining helper T cells and killer T cells, respectively—were once considered mutually exclusive. But "double positive" CD4+-CD8+ T cells have lately been discovered in people in various states of health. In a May study published in the *Journal of Experimental Medicine*, researchers led by Ludwig MSK's Jedd Wolchok and Taha Merghoub reported their findings on the biology of such double positive (dp) T cells isolated from murine and human melanoma and lung tumors. They showed that the dpT cells are tumor reactive and arise from predecessors that initially expressed only one of the markers, but began expressing both upon antigen stimulation of their T cell receptors (TCR). Upon such stimulation, predecessors of CD4+-derived dpT cells step up expression of a gene that establishes CD8 identity, and other genes involved in the destruction of their cellular targets. CD8+-derived dpT cells, meanwhile, begin expressing the regulatory CD4+ T cell-associated gene FoxP3. DpT cells from lung cancer and melanoma patients who responded to checkpoint blockade therapy proved to be potent destroyers of cancer cells in *ex vivo* assays and bore molecular markers of such capability. Taha, Jedd and their colleagues note their findings could eventually be used to identify T cells that target cancer cells and to generate polyfunctional T cells for various immunotherapies.

Tumor-induced double positive T cells display distinct lineage commitment mechanisms and functions | *Journal of Experimental Medicine*, 2022 May 23 Epub

Jedd Wolchok



Taha Merghoub

Jonathan Weissman

Tyler Jacks

Analysis of lung tumor evolution reveals drivers of metastasis

A *Cell* paper published in May co-led by scientists including Ludwig MIT's Jonathan Weissman and Co-director Tyler Jacks reported a granular analysis of lung cancer evolution in a genetically engineered mouse model (GEMM), tracing the lineage of tumor cells in unprecedented detail. The study employed a GEMM of lung adenocarcinoma, driven by oncogenic Kras and loss of the p53 tumor suppressor, that faithfully mimics human tumor progression. The cancer was initiated in the GEMM by a virus, which also activated a CRISPR-based cell-tracing technology—a DNA barcode that changes discreetly with each cell division. This allowed the researchers to generate granular family trees of cells that ultimately seeded metastases. Their analysis revealed considerable diversity among subpopulations of tumor cells, which sampled diverse transcriptional states early in the course of tumor evolution before settling into one that conferred greatest fitness. Notably, the cells evolved mainly via inherited changes to their gene expression programs rather than mutation, and the fittest of such states came to dominate the tumor and to drive metastasis late in lung tumor evolution. The methods developed for this study can now be applied to study many other clinically relevant aspects of tumor evolution, like the development of drug resistance.

Lineage tracing reveals the phylodynamics, plasticity, and paths of tumor evolution | *Cell*, 2022 May 5 Epub

Laura Attardi

The Mettl3 epitranscriptomic writer amplifies p53 stress responses
Molecular Cell, 2022 May 4 Epub

Study identifies new mechanisms that boost p53 signaling and tumor suppression

A team led by Ludwig Stanford's Laura Attardi explored novel mechanisms by which the p53 protein, often called the guardian of the genome, regulates gene expression by looking for novel proteins that interact with the versatile tumor suppressor in response to cellular stressors. The researchers isolated these proteins based on their binding to p53 and identified them by mass spectroscopy. They reported in a May publication in *Molecular Cell* that METTL3—a key component of an mRNA-modifying protein complex known as M6A RNA methyltransferase complex (MTC) that helps regulate gene expression—amplifies p53 signaling induced by cellular stress. The researchers showed that METTL3 helps to stabilize p53 and promotes the expression of its target genes in response to DNA damage and oncogenic signaling by more than one mechanism. Indeed, p53 is relatively less stable and adept at inducing its target genes in cells that are deficient in METTL3. Laura and her colleagues also showed that METTL3 enhances the tumor suppressing activity of p53 in human cancer cells and in mouse models of cancer. Finally, their analysis of human cancer genome data supports a role for MTC in reinforcing p53 function in cancer.

Tony Letai

Kai Wucherpfennig

A potentially novel approach to improving the efficacy of NK cell therapies

Natural killer (NK) cells have lately become a hot focus of efforts to develop new immunotherapies. Researchers led by Ludwig Harvard's Tony Letai, whose team collaborated closely with the Ludwig Harvard Kai Wucherpfennig lab in this study, reported in an April issue of *Cell* a potentially novel approach to improving the efficacy of NK cell therapies. The new strategy described by Tony and his team builds on their discovery that NK cell-induced apoptosis of cancer cells occurs through a signaling pathway that involves the mitochondria. This mechanism—"mtApoptosis"— depends on the balance of pro-apoptotic and anti-apoptotic proteins in the mitochondrion. Whether a cell destroys itself depends on which way that balance is tipped. The researchers showed that targeting by NK cells tips the balance in favor of pro-apoptotic proteins and so primes cancer cells for mtApoptosis. Combining NK cells with a class of drugs—BH3 mimetics—that push the balance further in that direction led to the synergistic killing of cancer cells in culture and the suppression of tumor growth in mouse models of cancer. They also show that a method for screening drugs developed in Tony's lab, BH3 profiling, can be used to identify the BH3 mimetic drug most likely to augment NK cell killing of a given cancer.

Augmenting NK cell-based immunotherapy by targeting mitochondrial apoptosis | *Cell*, 2022 April 20 Epub

Judith Agudo

Quiescent cancer cells resist T cell attack by forming an immunosuppressive niche | *Cell*, 2022 April 20 Epub

Clusters of quiescent cancer cells drive resistance to immunotherapy in TNBC

Researchers led by Ludwig Harvard's Judith Agudo reported in an April paper in *Cell* that quiescent cancer cells congregate in clusters in primary tumors of triple-negative breast cancer (TNBC) and that these clusters play a central role in resistance to immunotherapy. The clusters, which express genes associated with chemotherapy resistance and metastatic capability, form a niche within the tumor microenvironment that is poorly infiltrated with immune cells and markedly starved of oxygen. An integrated spatial and single-cell gene expression analysis of this niche revealed the extensive activation of gene expression programs associated with hypoxia—regulated by the transcription factor HIF1α—in the cancer cells. The clusters are also infiltrated with tumor-supporting fibroblasts and dysfunctional dendritic cells, immune cells that ordinarily help orchestrate anti-tumor immune responses. The activation of hypoxic gene expression programs in cancer cells, Judith and her colleagues found, also promotes the terminal exhaustion of T cells and suppresses their killing of cancer cells. Judith and her colleagues propose that quiescent cancer cell clusters form reservoirs of immunotherapy-resistant cancer cells, and that their targeted disruption could improve immunotherapy against TNBC and thwart disease recurrence.

How two chromatin-modifying complexes influence the state of cancer cells preparing for metastasis

A study co-led by Ludwig MIT Co-director Robert Weinberg employed a CRISPR screen to identify genes that control the plasticity of cells undergoing epithelial–mesenchymal transition (EMT), a complex gene expression program employed by settled cancer cells as they acquire the motility and invasiveness that allows them to metastasize. But the switch from the epithelial to mesenchymal state isn't binary: EMT programs can induce a spectrum of cellular states between the two poles. Recent evidence suggests it is those in a "quasi-mesenchymal" state that are most likely to form metastatic outgrowths. Bob and his colleagues identified in an April paper in *Nature Cell Biology* two chromatin modifying complexes—PRC2 and KMT2D-COMPASS—that control EMT plasticity and have very different effects on cells. They show that loss of KMT2D-COMPASS pushes cells into a fully mesenchymal state, while PCR2 dysfunction pushes them into the middle state associated with enhanced metastasis. An examination of data from breast cancer patients showed that PRC2 loss is indeed associated with poor survival, supporting the hypothesis that the quasi-mesenchymal state is more conducive to metastasis. The findings have implications for therapies now under development that target PRC2 function, which in some types of cancers might promote metastasis—not quite the effect intended.

Robert Weinberg

Genome-wide CRISPR screen identifies PRC2 and KMT2D-COMPASS as regulators of distinct EMT trajectories that contribute differentially to metastasis | *Nature Cell Biology*, 2022 April 11 Epub

Ludwig Oxford's lively retreat

From Left, Ludwig Oxford Director Xin Lu with the organizing committee, Marie-Laure Foisneau-Bates, Francesco Boccellato, Yang Shi and Carol Leung.

Peter Ratcliffe, left, presenting Sudipta Ghosh the "Science Meets Art" prize.

For the first time since the COVID-19 pandemic started, the entire Ludwig Oxford Branch came together—in actual person—for a two-day meeting and program of talks that kicked off on June 13. The first day of the retreat, held at the Belfry Hotel in Oxfordshire, began with a keynote talk by Ludwig Oxford Visiting Professor Stephen Baylin on viruses, mitochondria, inflammation, aging and the evolution of the cancer epigenome. That talk, as sweeping as it was engaging, was followed by presentations by the five newest members of the Branch, beginning with Yang Shi, who discussed his lab's mechanistic studies of chromatin and RNA modifications, their biological effects and the translational insights now emerging from his team's discoveries.

Following Yang's presentation, leadership fellow Parinaz Mehdipour introduced her group's studies of epigenetic and epitranscriptomic regulation of anti-viral signaling in cancer, after which adjunct scholar Ellie Barnes gave an overview of the DeLIVER program for the earlier detection of hepatocellular liver cancer, which she leads and which involves several other Ludwig Oxford research teams. Ludwig Institute Member Stefan Constantinescu presented his work on the role of mutant calreticulin chaperone proteins in myeloproliferative neoplasms. Finally, senior group leader Helen Byrne spoke about mathematical approaches to the study of tumor heterogeneity and their use in the identification of biomarkers to aid clinical decision-making.

The second session began with a series of four-minute flash talks by junior researchers at the Branch—one nominated from each of the 12 Ludwig groups. This was followed by a talk on publishing papers by Colin Goding, who noted some common mistakes and offered tips for success. Barbara Marte, a senior editor at *Nature*, then gave the audience a behind-the-scenes glimpse of scientific publishing and shared what she looks for in selecting papers

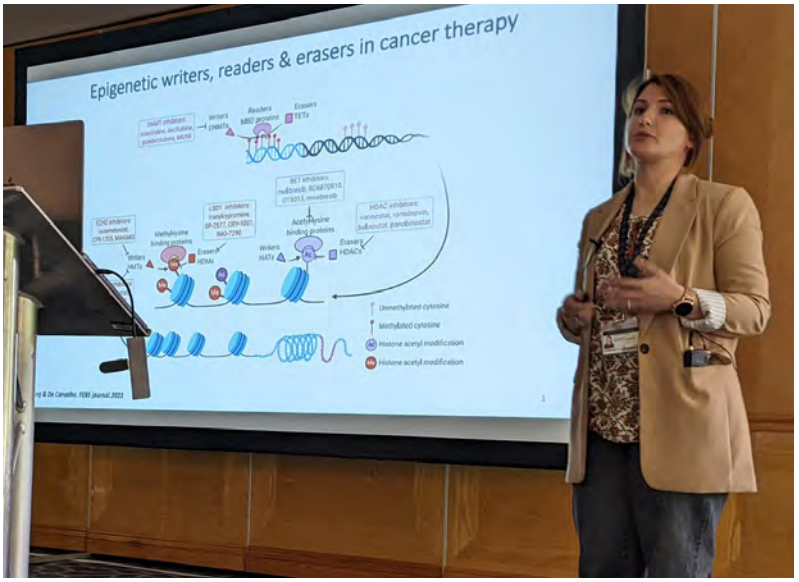
and Steve Mao, editor-in-chief at *Cancer Cell*, described his journal's publishing process and discussed the importance of both mechanistic and clinical studies in cancer research.

The day concluded with a poster session featuring 39 presentations from the Branch's students and post-doctoral researchers and a viewing of the "Science Meets Art" public engagement competition entries.

The second day kicked off with a presentation by Peter Ratcliffe on hypoxic signaling in cancer followed by a talk by Colin Goding on phenotypic heterogeneity in cancer and its link to cellular stress responses in melanoma. Benoît Van den Eynde shared an update on the clinical trial evaluating his group's novel cancer vaccine, VTP-600, in combination with anti-PD-1 checkpoint blockade for non-small cell lung cancer.

Skirmantas Kriaucionis presented his group's study of epigenetic mechanisms, including research into the effects of 5-hydroxymethylcytosine on transcription, and Chunxiao Song described the use of his TAPS method for simultaneous genetic and epigenetic DNA analysis and shared the latest data on detecting liver and pancreatic cancers using circulating tumor DNA. Benjamin Schuster-Böckler outlined his group's approach to exploring how cancer-causing mutations occur, and leadership fellow Francesco Boccellato discussed how understanding the body's response to chronic *Helicobacter pylori* infection could contribute to stomach cancer prevention.

Finally, Ludwig Oxford Director Xin Lu gave a talk on cellular plasticity in upper gastrointestinal cancers, including her group's investigation of the molecular switches involved in plasticity and the *ex vivo* models they employ. In her closing remarks, Xin reflected on the recent successes of the Branch and thanked the support team for their efforts in keeping research at the Branch going through the repeated lockdowns and challenges stemming from the pandemic.



Parinaz Mehdipour introduces her group's studies of epigenetic and epitranscriptomic regulation of anti-viral signaling in cancer.

Adjunct scholar Ellie Barnes gives an overview of the DeLIVER program for the earlier detection of hepatocellular liver cancer.

Visiting Professor Stephen Baylin delivers a keynote address on viruses, mitochondria, inflammation, aging and the evolution of the cancer epigenome.

PAT MORIN

Catalyzing cancer research

The Ludwig Institute's new deputy scientific director talks about his life, career, avocations—and his role.

We welcome Pat Morin, the Ludwig Institute's new deputy scientific director! A seasoned scientist and science administrator, Pat comes to us from the University of Pennsylvania, where he served as executive director for strategic alliances at the Abramson Cancer Center, overseeing interinstitutional collaborations for drug discovery.

After obtaining his PhD at Boston University in 1995, Pat joined the laboratory led by Bert Vogelstein and Kenneth Kinzler at Johns Hopkins University—before it became a Ludwig Center—as a postdoc, participating in studies on the activation of the Wnt/β-catenin pathway in colorectal cancer. In 1998, he started up his own lab as a tenure-track investigator at the National Institute on Aging, focusing on the molecular biology of ovarian cancer. After 15 years at the NIH, Pat moved over to the American Association for Cancer Research, where he served as senior director of scientific review and grants administration. In that position, he played a leadership role in the Stand Up to Cancer Initiative—working closely with large teams of scientists at multiple institutions—before joining the Abramson Cancer Center in 2016.

Pat will work with the Institute's Scientific Director Chi Van Dang and CEO Ed McDermott to, among other things, direct research activities, manage collaborative research and review scientific staff. *Ludwig Link* spoke with Pat in early July about his career, avocations and, of course, his new job. Below is an excerpt of that conversation.

Could you tell us a little about yourself and how you initially became interested in science?

I grew up in Quebec, Canada and became interested in science at a very young age. At home we had a few bookshelves with all

sorts of books in it, but somehow, I always gravitated towards the science books. In particular, I remember there was a TIME-LIFE book series on various aspects of science that I must have gone over dozens of times. There was one named *The Human Body* and another one named *Health and Diseases* that I found fascinating. My dad worked at a rubber manufacturing company and was able to bring me discarded laboratory equipment, such as flasks and pipets, and I set up a small laboratory in our basement. I enjoyed just sitting there and mixing random liquids pretending I was curing various diseases. One time, I put a bunch of grasshoppers in a cardboard box for future experiments, but during the night the grasshoppers escaped and invaded the whole basement. This led to the premature and permanent closing of my first laboratory. Later, when I was perhaps 12 or 13, I started subscribing to a French monthly magazine called *La Recherche*, similar to *Scientific American*, but a bit more technical. I remember being fascinated by the articles in this magazine, particularly those that had to do with molecular biology and theoretical physics. In the end, I chose biology, which I think was a very wise decision for me.

How did you wind up starting your research career at Johns Hopkins in the Vogelstein-Kinzler lab, and what did you work on while there?

I got my PhD working in Tom Gilmore's laboratory at Boston University, where I focused on basic mechanisms of NF-kB regulation. I really wanted to work on something more translational and just as I started looking for a lab for my postdoc, the Kinzler-Vogelstein lab published a series of exciting papers defining how mutations in mismatch repair genes were responsible for HNPCC, a colorectal cancer syndrome. This was extremely elegant scientifically, but also had enormous implications clinically. I applied for a postdoctoral position in the lab and got accepted. I did a lot of reading on HNPCC before joining the lab, expecting to work on that project, but Bert and Ken convinced me that working on the protein APC's mechanisms of tumor suppression would be a better fit for my background. We were first able to show that reintroduction of APC in colorectal cancer cells could cause apoptosis. Importantly, building on the molecular findings from our group, as well as others regarding the interactions and regulation of beta-catenin, we were able to identify, for the first time, activating beta-catenin mutations in colorectal cancer. Again, this had significant translational potential, and there are now several companies attempting to develop new compounds to inhibit the beta-catenin pathway in cancer.

> "I see myself as a science facilitator, a catalyst. There is a huge amount of exciting work being done by the Ludwig research community."

From a 10,000-foot level, what would you say are the main highlights of your research career? What are you most proud of?

Identifying beta-catenin mutations in cancer was certainly a huge highlight for me and still my most cited paper. It was an exciting time, and I believe it was an important contribution to our understanding of colorectal tumorigenesis. Later, in my lab at the NIH, we studied ovarian cancer and I would say that I'm particularly proud of publishing one of the first gene expression profiling studies in ovarian cancer. Back then in 2000, gene expression profiling was far from routine, like it is today, and it was a technical accomplishment to be able to get a fairly accurate portrait of thousands of genes expressed in ovarian cancer. From that study, we identified claudin proteins as highly expressed in ovarian cancer.

This represented one of the first demonstrations of claudin protein expression in human cancer, and we spent several years trying to unravel the mechanisms and roles of claudins in ovarian cancer. One of these proteins, Claudin-6, is actually being looked at as a possible target in CAR T-cell therapy of ovarian cancer. It's exciting, all these years later seeing that these findings are still relevant.

You eventually moved over to the AACR, where you worked on the Stand Up To Cancer (SU2C) awards? What did you find most engaging about that work?

When I was at the AACR, I oversaw the entire grants and scientific awards portfolio. We partnered with a large number of different organizations to award research funding and catalyze research all over the country and, as a matter of fact, all over the world. Knowing that my work could help accelerate research that could lead to breakthroughs in cancer treatment was definitely very satisfying. SU2C was certainly the highest-profile project I worked on, and by far the most complex grant program I oversaw. The selection process was very intricate and included in-person meetings with the finalists. Once awarded, the oversight was thorough and involved regular meetings with the teams. But the most important aspect of SU2C was collaboration in my opinion. Everything was centered on the premise that collaboration accelerates discovery and therefore can bring new therapies to patients faster. This is a concept I truly believe in, and which I found the most engaging about the job. Moreover, overseeing this program involved a constant interaction with the funded scientists, the committee members, AACR colleagues and SU2C leadership, which was also very exciting.

Could you tell us about your work at the University of Pennsylvania, overseeing an interinstitutional collaboration for drug discovery? What do you think you learned most from that experience?

My job at UPenn was to catalyze new collaborations between Penn investigators and pharmaceutical/biotech companies for drug development. I think everybody would agree that collaboration between academia and industry is essential for drug development. However, I learned that in practice this can be difficult. The culture is quite different between academia and industry, sometimes with competing interests. Intellectual property issues would almost always delay and sometimes prevent great ideas or projects that we had planned. I don't know that I necessarily have an answer to these complex problems, but what I learned is that it is very important to make everything very clear upfront: What are the goals? What are the terms? Who will do what? If it doesn't look like a win-win, a project that both sides are excited about right from the beginning, it probably won't work.

How do you envision your role here at Ludwig?

My experience so far has been that, as you fully assume a new position, you discover additional layers of responsibilities, things that you hadn't necessarily envisioned when you first started. But right now, in addition to the general oversight of the various programs, I see myself as a science facilitator, a catalyst. There is a huge amount of exciting work being done by the Ludwig research community and certainly significant opportunity for collaboration. But investigators are busy with their day-to-day work, which can be all-consuming. I believe that's where I can play a role, in identifying collaboration opportunities and then taking on the responsibility in making these projects happen.

What do you like to do most in your spare time? Any interests or hobbies you'd like to share with us?

I have played guitar since I was a kid and have been in many bands over the years, including

"Wild Type," the band that we started when I was in the Kinzler-Vogelstein lab. We played at various scientific meetings and various events to raise money for research—and had a lot of fun. I also love home improvement. I always have a project around the house. Owning a 100-year-old Tudor makes this hobby almost a necessity, but I enjoy it. Finally, I really enjoy wine. This interest goes back many years, but during the pandemic I decided to take the plunge, and I got my level three sommelier certificate.

Do you have a favorite band?

I've had many different favorite bands over the years, different phases I went through, like Led Zeppelin, Rush and R.E.M. However, the one constant band over all these years, a band I can always go back to and enjoy, is the Beatles, so I would have to say my favorite band overall is The Beatles.

Who's your favorite author, if you have one?

On the more classical side, I would say Charles Dickens. I just like the way he crafts his stories and the way he writes generally. In terms of contemporary authors, I really like Ken Follett. I first discovered him when I read *The Pillars of the Earth* years ago and have enjoyed his novels ever since.

Do you cook? What's your favorite cuisine?

I do cook every so often, but my wife is a wonderful cook (in addition to being a great scientist), so she typically takes the lead. I'm more like the sous-chef usually. My favorite cuisine is definitely Italian cuisine, and I could probably eat it every day for the rest of my life, especially if I could pair it with great Italian wines.

If you could meet any historical figure—from any walk of life—whom would you most like to meet?

Wild Type, circa 1996. Clockwise from left: Bert Vogelstein, Ken Kinzler, Bob Casero, Chris Torrance, Ellie Carson-Walter and Pat Morin.

Easy question for me. I have always been fascinated with Abraham Lincoln. Someone who rose from complete poverty, had only one year of formal schooling, to become what many consider the best president this country ever had. He is someone who handled every situation with honesty and humility, someone with great leadership skills who could recognize talent and knew how to make everybody around him better. He was magnanimous and didn't seem to ever hold a grudge. I think a conversation with Lincoln would be fascinating. One of my pandemic readings was *Team of Rivals* by Doris Kearns Goodwin, and it tells the fascinating story of how Lincoln assembled and led his cabinet. I would recommend it to anybody interested in the civil war, Lincoln, or even generally in how to be a great leader.

Now that you have returned to the office/lab, what is the most significant change you made during the pandemic that has made you more effective at work?

The pandemic accelerated our transition to a paperless office. Working with files on the network drive or SharePoint facilitated collaboration with colleagues in Zurich and in the New York offices. The use of electronic approvals via Adobe Sign accelerated the approval process, improved traceability of the authorizations and saved document shipping costs.

ALEXANDER BORISSOV
Zurich Office

YIBIN KANG
Ludwig Princeton

The biggest improvement we made during the pandemic is the digitalization of lab operations, including various channels in Microsoft Teams for scientific discussions and lab social, OneNote notebooks for project management with individual lab members or focused groups, and making lab notebook, equipment signup calendar and mouse colony management fully digital.

Pandemic and work from home during the periods of lockdown focused attention on efficient ways of using computing resources. I use Zoom/ Teams to communicate with people, and I am comfortable with remote-accessing electronic lab-books and data files, which helps me to keep in touch with lab activities when I am away from the lab.

SKIRMANTAS KRIAUCIONIS
Ludwig Oxford

The COVID-19 outbreak has taught us many lessons. Now that I have returned to the office/lab, I have tried to establish a better work-life balance, taking care of my mental wellbeing and taking time for self-reflection. This has increased my effectiveness, organizing my time to enable me to make the strongest contribution. We have learned to create a more compassionate workplace to increase the sense of belonging and commitment to our shared aims and objectives.

KHATOUN AL MOUSSAWI
Ludwig Oxford

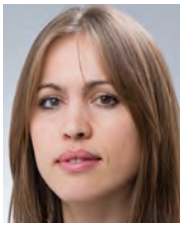
STAN NG
Ludwig Oxford

My key role for the return of people to work has been to create an effective and as safe a working environment as possible. To achieve this the Branch Administrator and I worked to assess, design, and implement COVID-safe working practices for all aspects of work at Ludwig Oxford. These plans allowed us to return to work sooner than many other units in our building.

MIKAËL PITTET
Ludwig Lausanne

We've realized that access to a great presentation, or a discussion with a colleague, is literally at our fingertips. Let's be clear: virtual meetings are far from a universally ideal mode of communication—they certainly lack a human touch—but when used wisely, they are an incredibly effective way to connect with the scientific world.

Opening a lab right before the pandemic was very challenging. Thus, optimizing my time distribution over meetings and "personal" working time (writing grants and papers, reading, etc.) is still critical. Prioritizing what is important and not just what is urgent is one of the most important lessons for optimal time management.



DENARDA DANGAJ
Ludwig Lausanne

I am starting my independent lab and can't imagine what it would be like without the tools I developed to work and interact remotely during the pandemic. Before the pandemic, I had never used Zoom. This week I participated in a monthly conference call for an international collaborative group, interviewed a technician candidate in Lebanon and spoke to a sales rep about buying a new piece of equipment.

ADAM WOLPAW
Ludwig Wistar

GREGORY BAKER
Ludwig Harvard

Many of us have increasingly relied on office messaging programs for managing scientific collaborations from a distance during the COVID-19 pandemic. My work efficiency has significantly benefited from the ability of these programs to organize and store important conversations and documents I have exchanged with my experimental and computational collaborators.

While it is obviously great to work again from the office and to have regular in-person meetings with my team members and colleagues, one important change I have implemented is to reserve enough 'time with no meetings' on my agenda that I can dedicate for writing (grants, papers, etc.).

MICHAL BASSANI-STERNBERG
Ludwig Lausanne

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