IN THIS ISSUE

6  |  Lausanne begins cancer vaccine trials
Three groundbreaking studies focus on dendritic cell vaccines

20  |  The inspired neuro-oncologist
Michelle Monje on her research and supporting women in science
LETTER

Welcome to the spring issue of Ludwig Link! We have a bumper crop of research news for you. Read on to learn about the publication of a long-sought protein structure (see cover) and what it reveals about how a mutant signaling protein drives cancer, how a routine dietary intervention improves therapeutic responses in mouse models of pancreatic cancer and the identification of a potential vulnerability in drug-resistant neuroblastoma. And that’s just a small sample of the features in our research news section.

We also have reports on Ludwig’s presence at the 2022 Annual Meeting of the American Association for Cancer Research, where five Ludwig-affiliated researchers were honored with election to the AACR Academy, and another received a major award.

Our interview in this issue is with Ludwig Stanford investigator Michelle Monje, who has lately won a string of awards and honors for her trailblazing work on pediatric and adult brain cancers. Michelle spoke with us about some of those studies, and how best to support greater gender equity in biomedical research.

Finally, to commemorate International Women’s Day in March, we launched a special #AskAScientist social media campaign asking Ludwig leaders about their support for gender equity and their advice to young researchers. See what they had to say on page 28.

Happy reading!

Sincerely,

Rachel Reinhardt
Senior Vice President for Communications

On the cover: A representation of the long-sought protein structure of Janus kinase (JAK), solved by Ludwig Stanford’s Christopher Garcia and colleagues (see Page 9). Upon cytokine (yellow) binding to its receptor (red), the JAKs (blue), attached to the cytoplasmic tail of the cytokine receptor (red), meet at a region in their middle in order to activate each other. This region contains mutations known to cause blood cancers through cytokine-independent JAK signaling.
Awards and distinctions

FOR LANDMARK DISCOVERY

Five Ludwig Cancer Research-affiliated scientists were elected Fellows of the Academy of the American Association for Cancer Research (AACR), class of 2022: Ludwig Oxford’s Yang Shi, Ludwig MSK Director Alexander Rudensky, Ludwig Stanford investigators Christopher Garcia and Crystal Mackall and Ludwig Scientific Advisor Victor Velculescu. The AACR notes that election to the Academy recognizes scientists whose research has “propelled significant innovation and progress against cancer.” Yang was recognized for his landmark contributions to epigenetics, including the demonstration that histone methylation is reversible and dynamically regulated, and the discovery of the first histone demethylase, LSD1. Crystal was honored for her prolific work in immunology and immunotherapeutics, especially the development of CAR-T cell therapies, and her translational research in pediatric oncology. Chris was recognized for his singular contributions to structural biology, most notably the first structure of a T-cell receptor bound to a peptide-MHC complex and that of many immunoregulatory proteins. Alexander was honored for his foundational discoveries on the biology of regulatory T cells (Tregs) and analysis of their wide-ranging roles in tumorigenesis and the suppression of autoimmunity. Victor, meanwhile, was recognized for his many contributions to our understanding and analysis of the cancer genome and the development of non-invasive cancer detection technologies. Our congratulations to all.
Ludwig Harvard’s Joan Brugge during a panel discussion at the AACR Annual Meeting, where she received the award.

FOR WORK ON WOMEN’S CANCERS

Ludwig Harvard Co-director Joan Brugge was one of five women scientists awarded The Victoria’s Secret Global Fund for Women’s Cancers 2022 Meritorious Awards, in partnership with Pelotonia and AACR. The award recognizes five female researchers who have made pioneering contributions to the understanding or treatment of breast cancer or any type of gynecologic malignancy. Joan was honored for her many discoveries elucidating the mechanisms of cancer initiation, growth and therapeutic resistance, work that has contributed to the development of new therapeutic strategies to overcome cancer drug resistance. The award took special note of her landmark isolation and characterization of the Src oncogene and her development of three-dimensional cell culture models for cancer research. Award recipients receive a $100,000 honorarium and are asked to nominate three to five outstanding investigators to be considered for grants in a women’s cancers research program. Joan and other honorees will serve as mentors to the researchers ultimately selected. The award was presented at the AACR Annual Meeting 2022, April 8-13, in New Orleans.
In collaboration with the Department of Oncology UNIL-CHUV, the Ludwig Lausanne Branch has launched two clinical trials of novel cancer vaccines, with a third nearing regulatory approval by the local Swissmedic authorities. All three trials are evaluating dendritic cell (DC) vaccines, in which dendritic cells obtained from a patient are cultured, loaded with neoantigens—the novel antigens, specific to each patient’s cancer, that are generated by random mutations across the cancer genome—and then reinfused into patients for therapy. Dendritic cells then present the neoantigens to T cells, activating a highly targeted immune response against each patient’s cancer.

The first trial, which started last year, is named PEP-DC and will examine the ability of personalized dendritic cell vaccines to prevent the recurrence of pancreatic cancer. The phase 1b trial will enroll 10-12 patients who have undergone surgery and have either no detectable cancer or a low burden of disease. They will all be treated with standard of care therapy plus immunotherapy and the personalized DC vaccine. A pair of patients have already received their personalized vaccines. The second trial, named LUNGVAC, started in December 2021. It is expected to ultimately enroll 16 patients with advanced or metastatic non-small cell lung cancer who will receive standard of care along with the personalized DC vaccine. The objective is to determine whether the vaccine amplifies responses to therapy. One patient enrolled in LUNGVAC has already received the personalized DC vaccine.

The trial now in the final stages of review by Swissmedic is a phase 1/2 trial of two types of DC vaccines for patients with
Clinical trials

A PRIME BOOST FOR IMMUNOTHERAPY

In January, the first patient enrolled in the MAGE trial received the first dose of VTP-600, a cancer vaccination strategy based on preclinical research led by Ludwig Institute’s Benoît Van den Eynde. VTP600 is being tested in patients with non-small cell lung cancer (NSCLC). The prime-boost vaccination delivers NY-ESO-1 and MAGE-A3, which are cancer antigens that were characterized and developed by Ludwig researchers. The antigens are carried by two types of viral vectors, ChAdOx1, a chimpanzee cold virus also used in the AstraZeneca COVID-19 vaccine, and MVA, used for smallpox vaccination. The phase I/IIa trial is testing the safety and initial efficacy of VTP-600 and is expected to enroll 86 people newly diagnosed with NSCLC. Patients whose cancers express MAGE-A3 will receive a priming shot of ChAdOx1-MAGE-A3-NY-ESO-1 vaccine, followed by an MVA boost delivering MAGE-A3. Those whose tumors express both MAGE-A3 and NY-ESO-1 will get the same prime vaccine followed by a boost using MVA-MAGE-A3 and MVA-NY-ESO-1. The vaccine will be tested in combination with the current first line treatment for NSCLC—chemotherapy and anti-PD-1 checkpoint blockade. Cancer Research UK’s Centre for Drug Development is managing and providing funding for the trial. Vaccitech Oncology Ltd. (VOLT), a strategic collaboration between Vaccitech and Ludwig, is supplying the VTP-600 vaccines.

Benoît Van den Eynde
Ludwig Institute

high-grade serous ovarian carcinoma. It will enroll 16 patients, half of whom will receive the personalized peptide DC vaccine, in which neoantigenic peptides identified in each patient’s cancer are used to pulse dendritic cells to prepare the vaccine. The other half will receive a vaccine prepared with dendritic cells that have been pulsed with whole tumor extracts obtained from the patient and processed using a method under development at Ludwig Lausanne to improve vaccine immunogenicity. They will also receive the PEP-DC vaccine. Both cohorts will additionally receive maintenance treatment of low-dose chemotherapy after being treated with the initial standard of care therapy.

Ludwig Lausanne’s Lana Kandalaft, who also leads the CHUV’s department of oncology’s Center for Experimental Therapeutics, is overseeing the clinical trial program. The vaccine preparation and the immunologic analysis of patient responses is conducted by Ludwig Lausanne’s Human integrated Tumor Immunology Discovery engine (Hi-TIDe), led by Branch Director George Coukos. The immunopeptidomics group of the Hi-TIDe, led by Michal Bassani-Sternberg, is responsible for identifying and prioritizing neoantigens for personalized vaccine preparation, while the T cell discovery group, led by Alexandre Harari, will be monitoring the immune responses elicited by vaccination in the three trials.
News roundup

**EPIC DEDUCTIONS**

Researchers led by Ludwig Stanford’s Ash Alizadeh and Maximilian Diehn described in *Nature Biotechnology* an epigenomic feature of cell-free (or cf) DNAs linked to their fragmentation patterns and predictive of how avidly individual genes they encode are expressed. Fragmentation patterns have previously been shown to be reliable indicators of the tissues or tumors from which cfDNA has been released. Those patterns can also be used to infer the expression levels of genes encoded by DNA, which can reveal the subtype and other biological features of the tumor the DNA came from—all of which could guide individualized therapy. Existing methods, however, require large amounts of DNA for such inference, and cfDNA is typically found at very low levels in blood. In their March publication, whose lead author is Ludwig Stanford scientist Mohammad Shahrokh Esfahani, Ash, Max and their colleagues described a new method, "epigenetic expression inference from cell-free DNA-sequencing" (EPIC-seq), that can predict the previous expression levels of genes encoded in cfDNA. Analyzing 329 blood samples from 201 cancer patients and 87 healthy adults, the researchers applied EPIC-seq to identify subtypes of lung carcinoma and diffuse large B cell lymphoma. They also showed that gene expression patterns predicted by EPIC-seq correlate with clinical responses in patients treated with anti-PD-1 checkpoint blockade. The researchers argue the method could be useful for both cancer diagnosis and the management of therapy.
ESSENTIAL IDENTITY

In a March paper in *Bioinformatics*, Ludwig Lausanne’s Massimo Andreatta and Santiago Carmona describe scGate, a tool to purify a cell population from complex scRNA-seq datasets based on marker genes identified in the scientific literature. scGate, which permits the automatized purification of cell populations with the use of a few marker genes or sets of such genes organized in a hierarchical structure, does not require reference gene expression profiles or training data. It outperforms the best available single-cell classifiers and can be applied to data collected using multiple technologies, including ATAC-seq and CITE-seq, significantly expanding its utility. Technologies like scGate can help researchers identify subtly different but vitally important—and targetable—functional states assumed by cells once believed to be largely uniform in their behavior, such as immune cells commonly associated with tumors.

STICKY PROBLEM

In a March paper in *Science*, Ludwig Stanford’s Christopher Garcia reported the long-sought structure of a Janus kinase, whose mutation drives blood cancers known as myeloproliferative neoplasms. The structure reveals the mechanism by which Janus kinase-1 (JAK-1) transmits signals, as part of a JAK-STAT signaling complex, when activated by cytokines and how a mutation (V617F) switches JAKs on uncontrollably to cause cancer. The JAK protein is attached to the cytoplasmic tail of the cytokine receptor. Each cytokine protein binds two of these receptors, juxtaposing their attached JAKs and prompting them to activate one another. The new structure shows that the co-activating JAKs meet at a flattened region in their middle. The change induced by the V617F mutation to JAK creates a sort of ball-and-socket connection between the two that makes them adhere far more firmly to one another, switching them on even in the absence of an activating cytokine to transmit growth signals. The finding opens the door to developing targeted small molecules that selectively disrupt the mutant JAKs. Though Chris’s team solved the structure of activated JAK-1, and the V617F mutation is found in JAK-2, the two are sufficiently alike to share a signaling mechanism.
**INTRINSIC TARGET**

Diffuse intrinsic pontine glioma (DIPG) and other H3K27M-mutated diffuse midline gliomas tend to weave into brain tissue and are uniformly and swiftly lethal. Ludwig Stanford investigators Michelle Monje and Crystal Mackall reported in a February *Nature* paper promising findings from a phase 1 clinical trial testing a CAR-T cell therapy they devised in four patients with H3K27M-mutant DIPG/DMG. Michelle’s lab collaborated with Crystal’s to show in 2018 that CAR-T cells directed against the GD2 antigen expressed by such tumors could clear DIPG in mouse models. In the current trial, three of the four patients with advanced midline gliomas exhibited unprecedented clinical and radiological benefits from the therapy, and the anticipated side effect of brain inflammation could be managed with intensive care. Those who responded to the intravenous transfusions of GD2-targeting CAR-T cells received additional infusions through an Ommaya catheter threaded into the ventricular system of the brain, which also relieved potentially deadly pressure from the inflammation. The researchers subsequently reported at the AACR Annual Meeting in April that sequential intravenous and cerebroventricular delivery in another set of patients induced dramatic tumor regressions in the ongoing clinical trial. For more on the background story of this study, check out our profile of Michelle and the Q&A in this issue (page 20).

---

**THERAPEUTIC DIET**

Researchers led by Ludwig Princeton Director Joshua Rabinowitz reported in a February *Med* paper that the ketogenic diet—high fat, modest protein and very low carbohydrate—synergizes with chemotherapy to triple survival time compared to chemotherapy alone in rigorous mouse models of pancreatic ductal adenocarcinoma (PDAC). The ketogenic diet mimics fasting by reducing circulating glucose and depressing levels of insulin, a hormone that drives tissues and tumors to consume the sugar. Insulin is an important promoter of cancer growth, especially in pancreatic tumors, while glucose is a critically important fuel for cancer cell proliferation. In multiple experiments over many years, mice engineered to develop PDAC or implanted with tumors that resembled those seen in patients were fed either a normal carbohydrate-rich diet or a ketogenic diet and treated with a standard-of-care combination of chemotherapies—nab-paclitaxel (Abraxane), gemcitabine and cisplatin. The mice receiving the chemotherapy and ketogenic diet had deeper and more durable tumor regressions. While the therapeutic benefit did not require the immune system, only mice with intact immune systems were among the long-term survivors. Josh and his colleagues also conducted an intricate examination of how ketogenic diets affect the metabolism of PDAC tumors and identified mechanisms that might account for the therapeutic effect. An ongoing clinical trial (NCT04631445) is currently testing the strategy in PDAC patients.
A TENDENCY TO REACTIVITY

Severe immune-related adverse events (irAEs) occur in up to 60% of patients with melanoma treated with immune checkpoint inhibitors (ICIs), and there’s no way to tell right now who’s likely to develop such toxicities prior to ICI treatment. In a study reported in *Nature Medicine* in January, a team co-led by Ludwig Stanford’s Aaron Newman analyzed 93 pre- and early on-ICI blood samples and three patient cohorts to identify characteristics predictive of adverse immune reactions. They found that a marked abundance of activated CD4 memory T cells and a high degree of sequence diversity in the genes encoding T cell receptors, which recognize antigens, are associated with severe irAEs. Aaron and his colleagues hypothesize that patients with this profile either have a tendency to develop autoimmune diseases or already have autoimmune reactions that aren’t clinically apparent. As an initial test of this idea, the researchers performed a similar analysis of blood samples from people diagnosed with autoimmune disorders like lupus and inflammatory bowel disease. They found in these cancer-free patients the same pattern of high CD4 memory T cells seen in melanoma patients with irAEs from immunotherapy. Their findings have implications for better clinical management of ICI therapy.

TAPS FOR CANCER DETECTION

A study led by Ludwig Oxford’s Chunxiao Song and his Oxford colleague Shivan Sivakumar optimized the TAPS technology developed in Chunxiao’s lab for the mapping of DNA methylation for use on cell-free DNA (cfDNA). Such circulating cfDNA, a tiny proportion of which is shed by tumors in people with cancer, is the focus of efforts to develop liquid biopsies for early cancer detection, most of which have focused on the detection of mutations. Knowing the methylation state of cfDNA can, however, provide critical information unavailable in the DNA sequence alone, such as the likely location of a tumor. Chunxiao and his colleagues reported in a September paper in *Science Advances* their use of the optimized TAPS—named cfTAPS—on 85 samples from patients with hepatocellular carcinoma (HCC), pancreatic ductal adenocarcinoma (PDAC) and noncancer controls. Their results demonstrated that they could generate the most comprehensive cfDNA methylome to date using just 10 nanograms of cfDNA. They demonstrated that cfTAPS provides multimodal information about cfDNA, including its methylation patterns, the tissue from which it originated and DNA fragmentation. The integrated analysis of these epigenetic and genetic features enabled accurate identification of early HCC and PDAC.
News roundup

**CLONE MAESTER**

To study relationships between clonal populations of cells in complex tissues—a capability essential to studying things like tumor evolution—requires the labeling of cells with genetic barcodes, which can only be done in experimental systems. In a February paper in *Nature Biotechnology*, Ludwig Harvard’s Peter van Galen and Tyler Miller, a research fellow in Bradley Bernstein’s lab, reported a method and accompanying software to capture genetic variants from high throughput scRNA-seq platforms. Named MAESTER (for Mitochondrial Alteration Enrichment from Single-cell Transcriptomes to Establish Relatedness), the technology enables high throughput identification of clonal relationships in complex, primary human tissues using mutations in mitochondrial DNA that serve as naturally occurring barcodes. MAESTER is compatible with the most common high-throughput scRNA-seq platforms. This should make the tracing of clonal relationships between cells concomitant with single-cell gene expression analysis accessible to more research laboratories and applicable to a wider range of studies.

**BRIDGE TO SUCCESS**

Chimeric antigen receptor (CAR) T cells promote the destruction of cancer cells by recognizing target antigens using an antibody fragment that they’ve been engineered to express. Trouble is, not all antibody fragments (scFvs) employed in CARs are equally suited for such uses, and even well characterized and highly selective antibodies that are presumed ideal can fail when repurposed and put to the tumor-targeting test. In a January paper in *Scientific Reports*, Ludwig Lausanne’s Steven Dunn and colleagues described a new screening method to select antibody fragments for CARs that are less likely to fail such tests. The in vitro CAR library discovery approach links antibody-driven bridging of tumor and effector T cells with an informative and functionally relevant CAR activation reporter signal. This establishes early on that the selected antibody isn’t just recognizing its intended target, but that it is doing so in a manner that can trigger CAR-T cell function. Steven and his colleagues validated their methods by isolating novel scFvs that recognize the cancer antigen mesothelin. The results show that their functional screening method can enrich and identify antibody fragments well suited for use in CAR-T cells, including many that would have been missed by classical in vitro antibody screening technologies.
News roundup

BALANCE MAINTENANCE

Researchers co-led by Ludwig Princeton Director Joshua Rabinowitz reported in Nature Metabolism in January that, in mice, homeostasis—or the maintenance of physiologically appropriate balance—for some key circulating metabolites is maintained primarily through their mass-driven consumption, making energy and carbon dioxide, which is exhaled as waste, not through the active regulation of their production or consumption. The researchers administered essential amino acids, serine, alanine, citrate and 3-hydroxybutyrate into the blood of mice and traced their flux—the rise and fall of the molecules and their derivatives—using isotope labelling. An excess of these metabolites did nothing to alter their production. Instead, their consumption ramped up in linear proportion to any excess. This mechanism held across varying conditions—feeding, fasting and high- and low-protein diets—with the breakdown of internal proteins making up amino acid deficits during fasting. The findings indicate that despite the extensive regulatory machinery governing metabolic pathways, mammals achieve circulating metabolite homeostasis primarily through their mass consumption by cells.

DUAL DISRUPTION

One way to identify a molecular target for a cancer drug is by knocking out a gene in a cancer cell and seeing if that impairs or prevents tumor growth. But, quite often, more than one gene’s product can perform a critical function for a cancer driven by a particular oncogene, and such redundancies can be concealed by genetic screens that interrogate only one gene dependency at a time. Indeed, many key therapeutics target closely related genes—like CDK4 and CDK6, or MEK1 and MEK2—which is why they work. In a December Nature Genetics paper, a team led by Ludwig Harvard’s William Sellers reported a screen to identify dependencies on functionally redundant, or “paralogous,” gene pairs using the gene editing tool CRISPR-Cas9. They also described its application to investigate the effects of disrupting 3,284 genes, 5,065 paralog gene pairs and 815 paralog families. The researchers discovered that disruption of both DUSP4 and DUSP6 selectively impairs the growth of cancer cells driven by the common mutated oncogenic drivers NRAS and BRAF via the hyperactivation of a signaling protein named MAP kinase. Further, cells that are resistant to drugs that target the MAP kinase signaling pathway are especially sensitive to the loss of DUSP4 and DUSP6. Bill and his colleagues argue that technologies such as theirs can identify previously overlooked targets for cancer therapy.
CELLULAR REVIVAL

A team co-led by Ludwig Oxford’s Yang Shi reported in Nature Communications that the epigenetic enzyme LSD1 is an important modulator of T cell exhaustion and that its inhibition could significantly enhance anti-PD-1 immunotherapy. T cells that are unproductively activated by antigen over long periods, as they are in tumors, become lethargic and dysfunctional—a state known as "exhaustion." A subset of such cells, known as progenitor-exhausted T cells, can be reinvigorated by anti-PD-1 immunotherapy. Yang and his team show in their November paper that LSD1 loss or inhibition in mice significantly expands the progenitor exhausted pool of T cells in multiple mouse models of cancer, serving as a source of more specialized T cells with enhanced anti-tumor activity. They found that LSD1 physically interacts and inhibits TCF1, a regulator of gene expression essential to the progenitor-exhausted T cell identity. Inhibiting LSD1 with small drug-like molecules restores TCF1 function and promotes longer lasting responses to anti-PD-1 immunotherapy. Yang and his colleagues suggest that if biomarkers associated with this mechanism of resistance are identified, eligible patients could receive LSD1 inhibitors in combination with checkpoint blockade immunotherapy. They also suggest the TCF1-LSD1 interaction is a sound target for drug development.

HIGH-DIMENSIONAL ANALYSIS

The integration of many different channels of imaging data—highly multiplexed imaging—makes deep molecular analysis of single cells from human tissues and tumors possible. However, performing the necessary computation to transform whole-slide images into single-cell data is a daunting task. To address this, a team of researchers led by Ludwig Harvard’s Peter Sorger described in a November paper in Nature Methods a modular, open source computational pipeline named MCMICRO. Multiplexed imaging generates a richly layered picture of individual cells and their relationships to each other in tumors and adjacent normal tissue. MCMICRO makes it possible to determine the identities of these cells and the subtleties of their functional states as well, all in the context of their spatial distribution, generating data that is highly complementary to single-cell gene expression analysis. A diverse community of laboratories, including those involved in the Ludwig Tumor Atlas Network, maintains and develops MCMICRO. Documentation, source code and video tutorials are available at mcmicro.org, and help is available via the image.sc forum. Click here for an overview of MCMICRO.
News roundup

TARGET TGFβ

Ludwig MSK’s Jedd Wolchok and Taha Merghoub led a team, including researchers from MSK, New York and Brussels, to explore transforming growth factor-β (TGFβ) production and activity in stroma-poor colon and melanoma tumor models. TGFβ is a cytokine that has multiple roles in healthy and cancerous tissues. In the former, it can subdue proliferation and programmed cell death, while in certain cancers, such as melanoma and breast cancer, its signaling can promote metastasis and compromise immune surveillance. To complicate matters further, there are three subtypes of TGFβ, each with distinct patterns of expression. So far, the expression of TGFβ and its inhibition within the tumor microenvironment has mainly been investigated in stroma-rich tumors. Taha, Jedd and their Ludwig MSK colleagues Aditi Gupta and Sadna Budhu reported in a November paper in Communications Biology that myeloid and dendritic cells are the main source of TGFβ1 in colon carcinomas and both TGFβ1 and TGFβ3 in melanoma. Targeting TGFβ1 in colon cancer, and either TGFβ1 or TGFβ3 in melanoma, delays tumor growth—apparently by enhancing the activity of CD8+ T cells that target tumors. Their analysis suggests that isoform specific TGFβ inhibition in stroma-poor tumors can alter the tumor microenvironment in favor of anti-tumor immunity, and the researchers showed that its combination with immune checkpoint blockade improved tumor control in mouse models.
**News roundup**

**VICIOUS GANGS**

Extrachromosomal DNAs are free floating donuts of DNA found in nearly half of all types of tumors that encode multiple copies of oncogenes and play a major role in tumor growth, evolution and drug resistance. Researchers led by Ludwig Stanford’s Howard Chang reported in *Nature* in November that ecDNAs act as a collective, huddling in hubs of 10-100 in the nuclei of cancer cells. Howard and his colleagues discovered that this clustering enables a surprising phenomenon called "intermolecular gene activation," in which enhancer sequences from one molecule of ecDNA can amplify the expression of oncogenes on other ecDNA molecules, dramatically boosting oncogene expression in a manner never seen on chromosomes. The researchers report that the hubs are tethered together by the bromodomain and extraterminal domain (BET) protein BRD4, and that the BET inhibitor JQ1 breaks up the hubs and inhibits the expression of their oncogenes. They found that, in a MYC amplified colorectal cancer cell line, MYC oncogene is fused to a duplicated promoter that receives multiple enhancer signals and is thus expressed at extremely high levels. Their findings demonstrate that ecDNA hubs serve as units of oncogene function and cooperative evolution, and could be sound drug targets for cancer therapy.

**DUAL FUNCTION DRIVER**

A study led by Ludwig Oxford’s Colin Goding published in *Genes and Development* in November described how a transcriptional regulator called TBX2 plays a crucial role in senescence, a state of stable cell cycle arrest that can halt cancer initiation. TBX2 is a DNA-binding transcription factor—a regulator of gene expression—previously linked to transcriptional repression in embryonic development and senescence bypass and proliferation in cancer. But how it exerts its effects was unclear. Colin and his team found that TBX2 acts downstream of PI3K signaling, which is important for senescence bypass in melanomas driven by a mutated BRAF gene. In BRAF-mutated melanoma, TBX2 binds and is required for expression of E2F1, a key anti-senescence cell cycle regulator. The study vastly expands knowledge of the repertoire of genes bound and regulated by TBX2. It also provides a fundamentally different perspective on TBX2 function in senescence and development: rather than acting as a dedicated transcriptional repressor, it can also activate genes—repressing genes that block the cell cycle but maintaining expression of genes that promote cell cycle progression. TBX2 is thus a crucial regulator of cancer initiation and progression.
INFLAMMATORY MEASURES

Researchers led by Ludwig Scientific Director Chi Van Dang and Adam Wolpaw, a postdoctoral fellow in Chi’s lab and a pediatric oncologist at Children’s Hospital of Philadelphia (CHOP), reported in a February publication in the Proceedings of the National Academy of Sciences how the epigenetic states assumed by neuroblastoma cells influence the tumor’s visibility to the immune system. Neuroblastoma cells alternate between two such functional states: the mesenchymal state—associated with cells responsible for recurrent disease, metastasis and chemotherapy resistance—and the far more common adrenergic state. Working with cell cultures of this cancer, Adam, Chi and their colleagues found that cells in the adrenergic state are unresponsive to double-stranded (ds) RNA, while those in the mesenchymal state respond to it readily with inflammatory signaling that can provoke immune responses. When exposed to a molecule named poly(I:C)—a mimic of dsRNA currently being evaluated in clinical trials—the inflammatory responses of mesenchymal cells made them susceptible to killing by the immune system’s T cells. Mouse studies confirmed that tumors composed of mesenchymal cells were far more infiltrated with immune cells. The findings have implications for the development of new strategies to treat drug resistant neuroblastoma tumors, and such efforts are already underway and will be spearheaded by Adam at CHOP.
Conferences

LUDWIG AT THE AACR ANNUAL MEETING

Ludwig researchers showed up in force—virtually and (gasp!) in person—at the 2022 Annual Meeting of the American Association for Cancer Research, which took place in New Orleans this year. Aside from presenting new data in dozens of posters, many gave talks at educational and meet-the-expert sessions, various symposia and at the prestigious plenary sessions on two mornings.

The opening plenary session on Sunday, April 10, saw three Ludwig-affiliated scientists presenting their research, beginning with Ludwig Harvard’s Franziska Michor, who described her lab’s quantitative studies and mathematical modeling of tumor cell heterogeneity and evolution, and how such studies can be applied to overcome drug resistance. Franziska was followed by her Ludwig Harvard colleague Marcia Haigis, who presented her lab’s exploration of metabolic phenomena in cancer, including how the activity of certain metabolic enzymes suppresses anti-tumor T cells and how obesity alters the metabolism of immune cells in a manner that increases cancer risk. (Marcia gave a special shout-out to Ludwig at the end of her talk! Ludwig Link notes the appreciation is mutual.)

A third Ludwig-affiliated researcher, Ludwig Johns Hopkins’s Nickolas Papadopoulos, discussed the opportunities and challenges of using liquid biopsies for the early detection of multiple cancers. He described technical innovations that have dramatically improved the sensitivity of their liquid biopsies and a variety of scientific and medical issues associated with the Hopkins team’s groundbreaking development of the multi-cancer liquid biopsy CancerSEEK.

The clinical trials plenary that afternoon saw the presentation of some very exciting research by Stanford pediatric oncologist Robbie Majzner, who reported the results of an ongoing clinical trial led by Ludwig Stanford’s Crystal Mackall and Michelle Monje (also see interview, page 20, and feature, page 10). The talk described how repeated infusions of anti-GD2 CAR-T cells into the bloodstream and the cerebrospinal fluid as a treatment for H3K27M-mutated diffuse midline gliomas
induced remarkable regressions, clinical responses and survival benefits in young patients with these universally lethal cancers. A video of one of those patients—a five-year-old girl who had been almost immobilized by her disease now riding a skateboard—elicited rare, spontaneous applause from the audience.

On Monday, Ludwig Oxford’s Yang Shi presented his lab’s studies on how epigenetics influences the exhaustion of T cells essential to anti-tumor immunity and described how a class of epigenetic drugs known as LSD1 inhibitors can reinvigorate the immune cells to boost the efficacy of checkpoint blockade immunotherapy. Ludwig Chicago’s Sean Pitroda described his work, much of it done in collaboration with Director Ralph Weichselbaum’s team, on the use of integrated molecular subtyping to personalize radiation therapy for oligometastatic disease.

At the plenary session on Tuesday, Ludwig Chicago investigator Thomas Gajewski presented his team’s investigation of the factors that influence immune infiltration of tumors and the efficacy of immunotherapy, like gut microbes and variations in immune-regulatory genes. A bit later, Ludwig MIT’s Matthew Vander Heiden presented his team’s studies on how metabolic and nutritional constraints in different types of tissue influence the growth of tumors and where they tend to metastasize. His Ludwig MIT colleague Richard Hynes, meanwhile, discussed his lab’s examination of the unusual extracellular matrix (ECM) of tumor cells, how its component proteins influence cancer growth and metastasis and strategies for targeting the ECM for diagnosis and therapy. Finally, on Wednesday, Yibin Kang reported his team’s research on the crosstalk between key molecular signaling pathways, tumor cells and their stroma—or supportive tissue—in the formation of metastatic niches in the bone.

And all this is just a sliver of the exciting research—and new discoveries—shared in New Orleans by Ludwig researchers. Check out the following hyperlinks for a full list of Ludwig-affiliated presentations and posters at the AACR Annual Meeting.
Michelle Monje has by any measure had quite a year. In September, she was named a MacArthur Fellow—an honor colloquially known as the “genius grant”—the same month that she was named an Investigator of the Howard Hughes Medical Institute. The following month, she was elected to the U.S. National Academy of Medicine.

True to form, Michelle capped those accolades with more groundbreaking science: a landmark publication in Nature in February reporting the preliminary results of a phase 1 trial she led with Ludwig Stanford Colleague Crystal Mackall examining a CAR-T cell therapy they devised in four patients with H3K27M-mutated diffuse midline gliomas. Three of the four patients diagnosed with these invariably lethal pediatric brain cancers exhibited radiological and clinical benefits from the therapy,
Q&A

I knew that it was going to be a long and very difficult road filled with many obstacles, but I have always maintained a glimmer of hope that we might really be able to change the cruel course of this disease, to give children and their families more, good quality time, to someday even cure the cancer.

“...

including such things as a renewed ability to walk and chew food. Its side effects, which, due to the nature of the therapy, included inflammation in the parts of the brain involved by tumor and a potentially deadly brain fluid build-up known as hydrocephalus, proved to be reversible with prompt and intensive care. The ongoing trial is the culmination of some two decades of pioneering work led by Michelle—much of which is described in our profile of her—that has brought new hope to the families affected by these intractable cancers. Michelle, Crystal and their colleagues presented an update on these results at the AACR Annual Meeting, reporting that a trial delivering repeated infusions of a new dosage of CAR-T cells, both intravenously and through a catheter directly into the brain, resulted in dramatic tumor regressions and clinical improvements in another set of patients with these cancers. And this is just one of several recent groundbreaking studies to come out of her lab.

Ludwig Link caught up with Michelle to ask her about all this and more.

Congratulations on receiving the MacArthur Award and being named a Howard Hughes investigator, not to mention your election to the National Academy of Medicine. How has all this affected your life and work?

I feel so supported. I’m already in such a fortunate position here at Stanford and with Ludwig’s support. But this makes me feel like we can really expand the research program, take on high-risk, high-reward endeavors in the lab. And there’s a degree of validation that people think we’re doing the right kind of work. I feel a great deal of gratitude.

Could you tell us what the results coming out of these ongoing clinical trials of CAR-T cell therapy for H3K27M-mutant gliomas have meant to you?

I have been dedicated to improving outcomes for children and young adults affected by this horrific cancer of the brainstem and spinal cord since, as a medical student, I first encountered this disease, first watched a child—who we could not help—die from her brainstem cancer. I knew that it was going to be a long and very difficult road filled with many obstacles, but I have always maintained a glimmer of hope that we might really be able to change the cruel course of this disease, to give children and
Beyond brain cancers, it is becoming clear now that the nervous system plays a critical role in many cancers. We are also exploring how we might best target this therapeutically.

You recently had a preprint on COVID brain fog, which you showed is very similar to the cognitive impairment often called “chemo-fog,” a phenomenon you have studied extensively. Could you tell us about that study?

I’ve been studying the neurobiology of cancer therapy-related cognitive impairment for about 20 years now and, again and again, for cancer therapy-related cognitive impairment, the central mechanism is an inflammatory one. When I saw how very inflammogenic the SARS-CoV-2 infection was, even in relatively mild cases, I worried that we were going to start to see the kind of cognitive impairment that we see in some cancer patients. And, indeed, within months of the pandemic, it was clear this was happening. The syndrome is nearly identical to what is commonly called “chemo-fog”—impaired attention, concentration, speed of information processing, memory, executive function. My lab doesn’t work with infectious agents, so I reached out to people I had never met and was able to connect with.
Akiko Iwasaki, who is a thought leader in the virology and immunology of COVID and other respiratory infections. She’s been a wonderful collaborator. What we found supported exactly what we had hypothesized: a particular pattern of reactivity in microglia (resident immune cells in the brain) associated with white matter, and consequent effects that lead to the loss of myelinated axons in the subcortical white matter, even after very mild COVID (in experimental models and in line with what we are seeing in the human disease). I am hoping that we will find that the same kinds of therapeutic interventions that are useful for cancer therapy-associated cognitive impairment will prove to be useful in the cognitive impairment that occurs with long COVID.

**Your work on neural firing and glioma growth has really proceeded apace. Could you tell us a little about this work?**

We have found that neuronal activity regulates both low- and high-grade glioma growth and progression in powerful ways. This occurs both through paracrine factors (neuronal activity-regulated release of molecules that signal as growth factors to the cancer) as well as through bona fide electrical communication via neuron to glioma synapses. Beyond brain cancers, it is becoming clear now that the nervous system plays a critical role in many cancers. We are also exploring how we might best target this therapeutically. Which existing drugs that target neurotransmitter receptors and ion channels might affect cancer growth? And we’re finding some powerful modulators of tumor progression in drugs that we use all the time. Certain drugs, anti-epileptic drugs for example, powerfully inhibit glioma progression and, disturbingly, we are finding that some commonly used drugs—in specific tumor contexts—may accelerate growth. There is a clinical trial open that I’m leading within the Pediatric Brain Tumor Consortium targeting an enzyme, ADAM10, that is involved neuronal activity-regulated glioma growth and in establishing neuron-glioma synapses through its cleavage of a factor called neuroligin-3.
Q&A

The neuroscientist Kathleen Susman, whom you met as a freshman in Vassar College, was an early mentor of yours. How did she influence your life and career?

Oh, she changed my life because she put me on the path in science. I had always been interested in science and in becoming a physician, since I think I was in kindergarten. But I had a really discouraging experience in high school, a teacher who quite literally said to me, “It’s a rare woman who has a mind for science. Don’t worry about it, sweetheart,” which I took to mean, “Don’t even try.” When I went to Vassar, Kate Susman was assigned to me as my pre-major advisor. And I said to her, “Gosh, I wish I could be a doctor, but I don’t have a mind for science.” I’ve known Kate for nearly 30 years, and I’ve never seen her look angry except in that one moment, when she learned where I got that phrase from. She looked at me, and she composed herself, and she said, “Well, you had a bad teacher, and we’re good here, so we’re going to fix this. I want you to sign up for my biology class ... let’s give this a go.” And that was it. I ended up doing research in her neuroscience laboratory, and everything clicked from there. She was an incredibly important pivot point in my life.

Having had that experience, what would you say to your younger self or to somebody who had a similar experience?

Follow what you love, and don’t listen to the noise. That principle came up again later in my career when I was an MD PhD student and when I was a resident: I got a lot of unsolicited advice that there was no way to have family and a big career in science or medicine, that one has to choose. That did not prove to be the case for me, and I am grateful I had the chance to do both. I think the advice I would give to women is to just believe in yourself and do what you care about. Other people are often wrong about your capabilities.

And your mom was also successful professionally. Was she an inspiration to you? Did she have a big influence?

Oh, absolutely. I was and am so close to my mother. She’s always been my biggest
cheerleader and supporter. She started as a computer programmer in the sixties and made her way through the ranks at IBM. She's really quite impressive, and raised me on her own for most of my life, since I was about three years old. She's a great mom and showed me that it is possible to balance career and family well.

We've seen a lot of reports about this pandemic taking a disproportionate toll on young scientists. Have you noticed anything like this?
I am deeply worried that we are going to lose a generation of young scientists, both men and women, but especially women. A couple of my former trainees were in the process of starting their own labs at the beginning of the pandemic, and that is one of the hardest things that a scientist ever does. To have a pandemic hit at that moment, it could have been a fatal blow. I think it will take many years for many junior faculty to recover. I'm very worried that despite the Band-aids applied to the problem by institutions, we're going to lose people from science.

What can be done, do you think—not a Band-aid—that could help these early career researchers?
We really have to give these young scientists financial support to recover, to lengthen the runway for them, because it's all just so incredibly difficult and expensive to launch a research program. We have to give them almost a do-over. We need to help them recover from this incredible blow at a very vulnerable time.

Taking a step back then, what do you think are the biggest societal and professional barriers for women in science?
Something that is incredibly important and that affects all new parents, but women disproportionately to men, is how difficult it is to become a new parent during training or during early faculty years. I have four children, and I feel like I got through that not because of the rules, but despite them. When I was a postdoctoral fellow, I had the benefit of a mentor—who was invested in me and my success—providing funding for a research assistant so that my project could continue while I was on maternity leave, and so I could still mentor the research assistant and direct the project while my hands were full at home. In general, we don't give people sufficient maternity leave, we don't give people sufficient time for things like lactation, or provide affordable childcare to graduate students, postdocs and young faculty.

Something I do for everybody in my lab, in addition to the expectation that mothers will work from home until babies are on solids, is to pair new parents, men and women, with a research assistant. It is good for the research assistant to have an opportunity to do more science before going off to graduate school or whatever their next phase may be, it's great for the scientist who needs a pair of hands at the bench and to practice mentoring, and it's good for science because it keeps the projects moving forward. That kind of support can make a really big difference. We need to make that not an exception, but more the norm.
What is your aspiration for gender equity in your recruitments?

“Despite improvements in gender equity over recent years, right now there still aren’t enough women at the highest levels. We need the very best scientists, male and female, to drive forward discoveries.”

XIN LU
Ludwig Oxford

“While we steadfastly safeguard gender equity through our recruitment policy at the Lausanne Branch, I consider promotion and retention equally critical in developing parity. It is a source of personal pride that the absolute majority of our women leaders have pursued their career with us for over 10 years. I have a sense this means that they recognize themselves in the core values and intentional policies with regard to gender equality here in our Lausanne community.”

GEORGE COUKOS
Ludwig Lausanne

“Recruitments represent the chance for any research program to gain the diverse perspectives needed to break paradigms in cancer research. I aspire to recruit based on skills and ideas and also aim to fix recruitment imbalances that have been hard-wired into programs from past structural biases.”

GEORGE DEMETRI
Ludwig Harvard

“As the Ludwig Institute’s scientific director, I believe in creating career opportunities with equity being paramount. Gender equity is critically important to enhancing the diversity of ideas. Further, it encourages the next generation of women cancer researchers to join the cause and contribute to our evolving knowledge of cancer biology.”

CHI VAN DANG
Ludwig Cancer Research

“My aspiration is to have as many females as males in the recruitment, with equitable representation across minority groups as well.”

IRV WEISSMAN
Ludwig Stanford

“In the Ludwig Collaborative Lab at MSK, we strive to have a team that represents the very best of creative translational scientists spanning across genders and ethnicities. We celebrate International Women’s Day and affirm our belief that science should be an open field for limitless success and advancement of women.”

JEDD WOLCHOK
Ludwig MSK

“In my positions as a department chair, Ludwig Chicago co-director and assistant director of our cancer center, I have had numerous opportunities to recruit new faculty and have made every effort to achieve gender equity in each of these settings and will continue to do so in the future.”

GEORGE DEMETRI
Ludwig Harvard
What advice would you give your younger self as you started your career?

“Be prepared at times to feel as if you don’t belong. When this happens, it means that you bring something different to the table. This moment is THE most important time to stay.”

“Success of senior scientists is inspiring, but don’t measure yourself against scientists 10 or more years your senior. Choose role models who are close to you in age and stage of career.”

“Achieving gender equity and diversity is critical as it brings greater strength and creativity to cancer research, facilitating the transformative discoveries that benefit patients. Ensuring equal representation in the workplace, at the podium, in publications and in leadership requires attention and persistence. This should be incorporated into the missions of all our institutions. What you do to pave the way and break down barriers can make it easier for those that follow behind.”

“Don’t be afraid of taking risks, you never know where life will take you. You will encounter ups and downs in your career, which make you resilient. Follow your curiosity, and be passionate about your dreams. Never underestimate yourself.”

“Pay more attention to your own instincts than the expectations of others when making career decisions. Evaluate your priorities, and accept they will change over time. Seek counsel from people you respect and trust. Don’t dwell on ‘what if,’ and have the confidence to take a few risks.”

“Find a mentor early. Seek out one or two accomplished women who can be a sounding board as you navigate your career. When you achieve your goals, reach back and mentor someone else to help to build a powerful bench of influential leaders.”
Required reading

Click on the title to read an abstract of the study

### Ludwig Harvard
- **Nature Biotechnology**
  - 2022 February 24
  - Online ahead of print
  - Mitochondrial variant enrichment from high-throughput single-cell RNA sequencing resolves clonal populations.

### Scientific Reports
- 2022 January 21
  - A cell-based phenotypic library selection and screening approach for the de novo discovery of novel functional chimeric antigen receptors.

### Ludwig Lausanne
- **Bioinformatics**
  - 2022 March 8
  - Online ahead of print
  - scGate: marker-based purification of cell types from heterogeneous single-cell RNA-seq datasets.
  - Andreattta M, Berenstein AJ, Carmona SJ.

### Ludwig MSK
- **Communications Biology**
  - 2021 November 17
  - Isoform specific anti-TGFβ therapy enhances antitumor efficacy in mouse models of cancer.

### Ludwig Oxford
- **Genes & Development**
  - 2021 November 24 Epub
  - TBX2 controls a proproliferative gene expression program in melanoma.

### Nature Communications
- 2021 November 24
  - LSD1 inhibition sustains T cell invigoration with a durable response to PD-1 blockade.
  - Liu Y, Debo B, Li M, Shi Z, Sheng W, Shi Y.

### Ludwig Stanford
- **Nature Biotechnology**
  - 2022 March 31 Epub
  - Inferring gene expression from cell-free DNA fragmentation profiles.
Required reading

**Science**
2022 March 10
Online ahead of print

*Structure of a Janus kinase cytokine receptor complex reveals the basis for dimeric activation.*

**Nature**
2022 February 7 Epub

*GD2-CAR T cell therapy for H3K27M-mutated diffuse midline gliomas.*

**Nature**
2021 November 24 Epub

*ecDNA hubs drive cooperative intermolecular oncogene expression.*

**Ludwig WISTAR**
Proceedings of the National Academy of Sciences
2022 February 8

*Epigenetic state determines inflammatory sensing in neuroblastoma.*

Click on the title to read an abstract of the study