# LUDWIG LINK

JULY 2019

## IN THIS ISSUE



LUDWIG CANCER RESEARCH

LIFE-CHANGING SCIENCE

LUDWIG LINK
ludwigcancerresearch.org JULY 2019

## LETTER

Welcome to the summer 2019 issue of Ludwig Link. We have for you, as always, a rich assortment of news from the frontiers of Ludwig's cancer research.

You'll read in this issue about how miniature antibodies derived from alpacas, of all critters, hold significant promise for the diagnosis and treatment of metastatic cancers, a liquid biopsy's performance as a predictor of colorectal cancer relapse and how a uniquely targeted version of a blood pressure drug and, separately, a diabetes drug, could significantly improve responses to cancer immunotherapy. All that, by the way, is just a small sample of the notable Ludwig research reported in these pages.

Don't forget to check out the honors and awards received by the stellar scientists affiliated with Ludwig. Ditto for our Q&A (page 18). This time around it's with a scientist whose love for science really is in the blood. That would be Ludwig Stanford researcher Ravi Majeti, who has made major contributions to our understanding of stem cells and leukemia. Ravi also speaks about his passion for mentoring the next generation of physician-scientists, and basketball.

You'll also find in here a few pictures from the Ludwig reception at this year's AACR meeting. Finally, in our "Ask a Scientist" section, we have scientists weigh in on what they believe to be the biggest challenges facing cancer researchers and oncologists today. Check out their answers on page 24. See if you agree.

Happy reading,

Rachel Reinhardt
Vice President for Communications

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**On the cover:** Norma Masson of Ludwig Oxford

Ken Kinzler
Ludwig Johns Hopkins

## FOR TRANSFORMING CANCER GENETICS

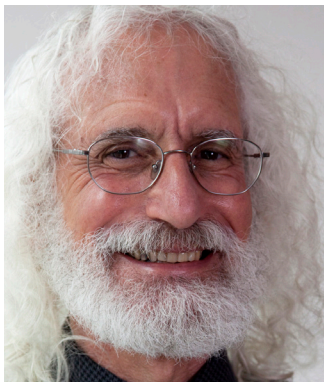
Ludwig Johns Hopkins' Co-director Ken Kinzler was named a member of the American Academy of Arts and Sciences (AAAS). Ken is renowned for his development of groundbreaking genomics and diagnostics technologies and his contributions to our understanding of the molecular genetics of dozens of cancers. Collaborating with Co-director Bert Vogelstein and colleagues at Ludwig Johns Hopkins, Ken played a central role in the identification of more than 20 cancer driver genes and the generation of the first maps ever made of all of the expressed genes—or exomes—in scores of cancers. Ken's inventions have been critical to the team's development of minimally invasive tests that detect rare fragments of mutated DNA shed by tumors into body fluids. AAAS membership recognizes individuals for their leadership, interdisciplinary work and "achievements in advancing the common good." Ken will be inducted at a ceremony in October in Cambridge, Massachusetts.

Cigall Kadoch
Ludwig Harvard

## FOR UNPACKING DNA PACKAGING

Ludwig Harvard's Cigall Kadoch received the American Association for the Advancement of Science (AAAS) Martin and Rose Wachtel Cancer Research Award, which honors early-career investigators who have performed outstanding work in the field of cancer research. Cigall's lab explores how structural changes to chromatin—the term for DNA in its protein packaging—alters the expression of genes. She discovered how a gene mutation to a component of a complex of proteins that remodels chromatin to regulate gene expression causes a rare, hard-to-treat cancer called synovial sarcoma. She and her colleagues have been applying the lessons learned from that model to understand how aberrant chromatin remodeling contributes to more common types of cancer, with the aim of devising new cancer therapies.

**Arlene Sharpe**
**Ludwig Harvard**



**Phil Greenberg**
**Ludwig Scientific Advisory Committee**

## FOR ADVANCING IMMUNOTHERAPY

Ludwig Harvard's Arlene Sharpe and Ludwig Scientific Advisory Committee member Phil Greenberg were elected to the 2019 Class of Fellows of the American Association for Cancer Research (AACR). Arlene has made major contributions to our understanding of the immunoregulatory pathways that control T cell responses, most notably the role played by CTLA-4 and PD-1 in countering T cell activation. Her work helped lay the foundations for immunotherapies for cancer, autoimmune diseases and transplant rejection. Phil is an authority on T cell therapies. He and his team were the first to show that it's possible to extract T cells from a patient, isolate and selectively expand those that target diseased cells and infuse them into the patient for therapy. His lab continues to engineer T cells to better target cancer cells and survive the rigors of the tumor's microenvironment.



**Francesco Boccellato**
**Ludwig Oxford**

**Pedro Moura Alves**
**Ludwig Oxford**

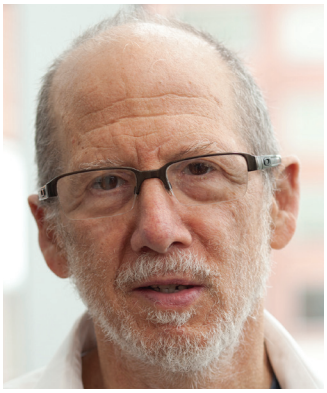
## NEW OXFORD FELLOWS

Ludwig Oxford welcomes two leadership fellows, Francesco Boccellato and Pedro Moura Alves—both alumni of the Max Planck Institute for Infection Biology in Berlin, Germany. They will join the Branch's exploration of the links between infection and cancer. Francesco specializes in generating 3D polarized epithelial culture models of the human gastrointestinal mucosa—the soft lining of inner body cavities—to study the effect of cancer-associated pathogens, such as the bacterium *Helicobactor pylori*. As these cultures resemble the mucosal barrier in situ, they are termed "Mucosoid cultures." Pedro's research background is in immunology, with an emphasis on the study of host-pathogen interactions via pattern recognition receptors, which are critical to the body's frontline defenses against infection and cancer. His biggest scientific discovery to date stems from his work on the aryl hydrocarbon receptor (AhR), which is involved in the recognition of bacterial virulence factors.

Pier Paolo Pandolfi
Ludwig Harvard

## DUAL PURPOSE PROMISE

Acute myeloid leukemia (AML) is a cancer of blood forming cells that primarily affects adults, with approximately 20,000 new cases in the US each year. Patients typically respond to chemotherapy, but more than half eventually relapse. About 20% of AML cases are driven by mutations to metabolic enzymes named IDH. In a June paper in *Cell Research* a team of scientists led by Ludwig Harvard researcher Pier Paolo Pandolfi revealed vulnerabilities in AML cases driven by mutations to IDH that can be targeted to overcome this drug resistance. The team demonstrated that a drug combination—arsenic trioxide (ATO) and all-trans retinoic acid (ATRA), a molecular relative of vitamin A—that, thanks to Pier Paolo's previous studies, is already in clinical use for the treatment of acute promyelocytic leukemia, works just as well in various mutant IDH1/2 mouse models of AML and human AML cells. The researchers plan to soon test the drug combination in clinical trials.



Bert Vogelstein
Ludwig Johns Hopkins

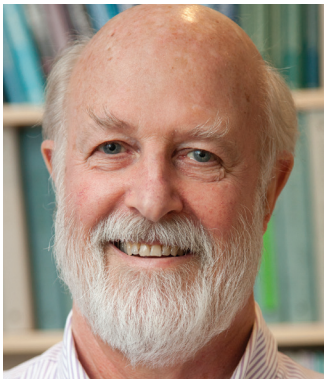
## EARLY WARNINGS

The postoperative surveillance of patients treated for colorectal cancer (CRC) is intended to prolong their survival by detecting and treating precancerous polyps and recurrent cancers while they're still at a curable stage. In a May paper in *JAMA Oncology*, a team led in part by Ludwig Johns Hopkins Co-director Bert Vogelstein evaluated whether circulating tumor DNA (ctDNA) levels capture disease recurrence earlier than conventional postoperative surveillance in patients with resected CRC. The team collected 319 blood samples from 58 individuals with non-metastatic stage I to III CRCs after they had undergone surgery at four Swedish hospitals over a nine-year period. ctDNA testing, they found, can predict disease recurrence. Blood samples were collected from patients one month after surgery and every three to six months thereafter for ctDNA analysis, with a median follow-up of 49 months. Among patients who tested positive for ctDNA, 77% developed recurrent disease, while none of the 45 patients who remained negative experienced a relapse. In the three patients who tested positive without relapse, ctDNA subsequently fell to undetectable levels during follow-up.

## NANOBODY, MEGAPOTENTIAL

A team led by Ludwig MIT researcher Richard Hynes has developed a new method to detect and target tumors based on the extracellular matrix (ECM), the meshwork of macromolecules in which both normal and cancerous cells are embedded. Certain proteins are abundant in the ECM of cancer cells but absent from healthy tissues. These proteins do not mutate as the cancer progresses, making them reliably detectable proxies for tumors. To detect these ECM markers, the researchers made use of "nanobodies," a type of antibody derived from alpacas. A tenth the size of typical antibodies, nanobodies penetrate more deeply into tissues and are more easily cleared.

The researchers developed libraries of nanobodies against the malignant ECM and picked one that binds to a variant portion of the protein fibronectin that is rarely seen in normal adult tissues. Nanobodies tagged with radioisotopes produced clear images of tumors and metastases in a mouse model. Richard and his colleagues note that the nanobodies could be similarly armed with drugs to generate targeted therapies for cancer. The findings were published in May in the *Proceedings of the National Academy of Sciences*. Another paper in the same journal in April showed that arming CAR-T cells with the nanobody could inhibit tumor growth in a mouse model.



**Richard Hynes**
**Ludwig MIT**

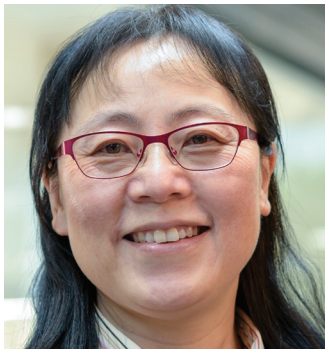
## SIGNATURE ANALYSIS

PARP inhibitors are used to treat cancers fueled by a defect in the cell's DNA repair machinery known as homologous recombination (HR) deficiency. They are typically given to breast cancer patients who have mutations in their BRCA gene, which is one cause of HR deficiency. But many types of cancer fueled by the defect, including those of the breast, lack mutations to the BRCA gene. In a May paper in *Nature Genetics*, a team led by Ludwig Harvard researcher Peter Park reports a new and much improved algorithm for detecting HR deficiency that could dramatically improve its detection if incorporated into existing clinical genetic tests. Named SigMA (for Signature Multivariate Analysis), the algorithm spots HR based on the overall pattern of mutations observed rather than looking for a specific driver mutation. Whereas previous methods required data from whole-genome or whole-exome sequencing, SigMA allows one to do the same analysis even on the gene panel data. The researchers say incorporating SigMA into standard gene tests could significantly broaden the pool of cancer patients eligible for PARP-inhibitor therapy.

**Peter Park**
**Ludwig Harvard**

## CAUTIONARY NOTE

Autophagy, a form of internal cannibalism by which cells recycle the components of their broken down and superfluous biochemical machinery, plays a complex role in cancer. Drugs currently under development target the process in tumors that are driven by an oncogene named Ras. In a May paper in *Autophagy*, researchers led by Ludwig Oxford Director Xin Lu studied the effect of autophagy inhibition on the epithelial to mesenchymal transition (EMT), a process of cellular transformation essential to cancer metastasis. Notably, they show that autophagy inhibition promotes EMT in tumors whose growth is driven by the Ras oncogene, and that this occurs due to the activation of another signaling pathway controlled by a protein named NF-kappa B (NFKB). The researchers detail the signaling circuits by which this activation occurs and show that its disruption suppresses the induction of EMT upon the inhibition of autophagy. They suggest NFKB inhibitors may need to be used in combination with autophagy inhibition for the treatment of Ras-mutated cancers.



Xin Lu
Ludwig Oxford

Ash Alizadeh
Ludwig Stanford

Aaron Newman
Ludwig Stanford

## HIGH-RES DECONSTRUCTION

Single-cell RNA sequencing (scRNA-seq) is a powerful technique for profiling the characteristics of individual cells in tissue based on gene expression profiles. It is, however, impractical for use on standard clinical samples. A team led by Ludwig Stanford researchers Ash Alizadeh and Aaron Newman reported in a May paper in *Nature Biotechnology* a computational technique to overcome this limitation. Named CIBERSORTx, the algorithm enables the profiling of individual cells in whole-tissue samples, datasets and even clinically preserved samples. The technique first analyzes gene expression in individual cells to generate "bar codes" for each of its cellular subtypes and their possible states. Those barcodes are then applied to the analysis of a whole tissue sample to profile the full spectrum and states of its constituent cells. The team analyzed over 1,000 whole tumors with CIBERRSORTx and demonstrated its use in identifying and profiling the individual properties of their cancerous and noncancerous cells. They also applied it to melanoma tumors and clinical datasets to confirm a hypothesis that higher levels of the proteins PD-1 and CTLA-4 on tumor-infiltrating T cells predict a better response to checkpoint blockade immunotherapies that target those molecules. The algorithm is available through an internet link.

## LIMITED RELEASE

The majority of cancers resist checkpoint blockade immunotherapy, in many cases because the tumor microenvironment actively suppresses immune responses. Among the factors responsible for this immunosuppression are cancer-associated fibroblasts (CAFs). In their active state, CAFs bar entry to the immune system's killer T cells, boost acidity and hypoxia inside tumors and secrete molecules that activate regulatory T cells—all of which suppress anti-tumor immune responses. In a May paper in the *Proceedings of the National Academy of Sciences*, a team led Ludwig Harvard researcher Rakesh Jain shows that combining a tumor-targeted version of an angiotensin receptor blocker (ARB), a blood pressure drug, with immune checkpoint blockade could help expand the use and efficacy of cancer immunotherapies. ARBs can switch CAFs from an active to a dormant state, but their use with immunotherapy has been limited by their physiological effect on blood pressure. Rakesh and his colleagues linked the ARBs to a polymer that degrades in the acidic microenvironment of solid tumors but not in the neutral environment outside them. These polymer-linked ARBs selectively release ARBs into tumors. When combined with checkpoint blockade, they ramp up anti-tumor immune responses and extend survival in mouse models of breast cancer, a type of tumor typically resistant to such immunotherapies.

**Rakesh Jain**
**Ludwig Harvard**

## A DANGEROUS TOLERANCE

Non-small cell lung cancers account for 85% of lung cancer diagnoses. Approximately 10% of these cancers carry a mutation to the epidermal growth factor receptor (EGFR), a protein on the surface of cells that transmits growth promoting signals. In an April paper published in *Nature Metabolism*, a team led by Ludwig Harvard researcher Frank Slack identified a novel pathway in EGFR-mutant non-small cell lung cancers that induces drug tolerance, a state of declining response to therapy that precedes drug resistance. They show that the reversible tolerance, induced in a subpopulation of cancer cells, is regulated by a microRNA—a small transcript of DNA that does not encode a protein but, rather, regulates gene expression. miR-147b generates a reversible state of tolerance to the EGFR tyrosine kinase inhibitor osimertinib. The researchers report that blocking the microRNA in patient-derived 3D cultures delayed the onset of tolerance to osimertinib. Frank and his team are currently testing approaches to targeting this new pathway in clinically relevant mouse models of EGFR-mutant lung cancer.

**Frank Slack**
**Ludwig Harvard**

Paul Mischel
Ludwig San Diego

## ACQUIRED DEPENDENCIES

A team led by Ludwig San Diego's Paul Mischel showed in a May paper in *Nature* how the tissue from which a tumor arises can have critical effects on its subsequent biochemical choices, establishing dependencies that can be exploited for new therapies. The researchers analyzed thousands of tumors and normal samples from 19 different tissue types and found that where a cancer arises is the major determinant of how its cells make the essential molecule NAD. Normal cells can make NAD by using any one of three pathways but, Paul and his colleagues showed, cancer cells become dependent via complementary mechanisms of gene amplification and epigenetic remodeling, which are shaped by tissue context. A key regulator of one of the three pathways, an enzyme named NAPRT, is highly expressed in tumors arising from tissues where the enzyme is elevated in any case. Such cancers tend to have multiple copies of the NAPRT gene or another gene, NADSYN1, essential to that pathway. Those arising from tissues where NAPRT is not elevated depend on another pathway to make NAD, and Paul and Ludwig San Diego's Bing Ren identified the epigenetic mechanism underlying this dependency. Tumors that depend on different NAD-making pathways could each be selectively shrunk in the same mouse by genetically or pharmacologically disrupting each tumor type's chosen pathway. Notably, that effect could be recapitulated with a compound that blocks NADSYN1. This study provides new insight into how tumor genotype and tissue context interact to determine the metabolic choices that cancer cells make and, with additional research, could contribute to a more informed precision medicine strategy for cancer patients.

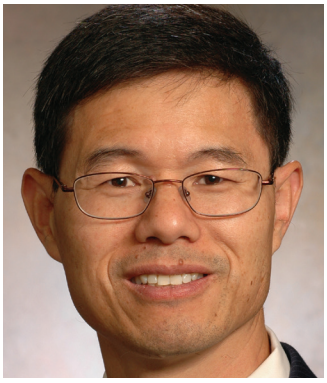
Benoît Van den Eynde
Ludwig Institute

## INSPECTION DETECTION

Certain immune cells, like dendritic cells, can eat up and digest telltale protein fragments associated with sick and cancerous cells and present them to killer T cells, which get stimulated to destroy cells carrying that antigen. The dendritic cell's processing of such fragments, or antigens, for "cross-presentation" is not very well understood but is known to occur via two pathways—the vacuolar pathway and the TAP-dependent pathway—that were thought to be mutually exclusive. In a paper in the *Journal of Immunology*, a team led by Ludwig's Benoît Van den Eynde reported things are not quite that simple. They described their examination of the processing of a long fragment of a cancer antigen named MAGE-A3 and the discovery of a new mechanism of antigen processing for cross-presentation that involves elements of both pathways. A better understanding of cross-presentation and its mechanisms is useful to the design of cancer vaccines.

## NEW NANOSTRATEGY

A team led by Ludwig Chicago researcher Wenbin Lin reported in an April paper in *Nature Communications* that colorectal tumors might be made susceptible to checkpoint blockade when the immunotherapy is combined with the systemic delivery of a specially designed nanoparticle. A chemical species known as a coordination polymer, the biodegradable nanoparticle has unique chemical properties that permit its use for the simultaneous delivery of drugs—in this case the chemotherapy oxaliplatin and the antimalarial dihydroartemesinin—that have divergent chemical and physical properties. Delivered together, these drugs synergize to generate reactive oxygen species (ROS) that kill cells in a way that stimulates a brisk immune response. Combined with PD-L1 blockade, the nanoparticle dramatically boosted the effects of immunotherapy in a mouse model of colorectal cancer, which typically resists immunotherapy. The combination therapy prompted an immune response of sufficient robustness to instill an immune memory of the cancer in treated animals: mice cured of their colorectal tumors with the nanomedicine rejected colorectal cancer cells that were subsequently transplanted into them. The researchers are preparing to test the nanoparticle in a clinical trial.



**Wenbin Lin**
**Ludwig Chicago**

## ROGUE CHAPERONE

Myeloproliferative neoplasms are a family of slow-growing blood cancers that can progress to more acute malignancies. They are driven by the abnormal activation of the thrombopoietin receptor (TpoR). Two myeloproliferative neoplasms—essential thrombocythemia, in which the body produces too many platelets, and myelofibrosis, where scarring of the bone marrow is induced by hyperproliferation of blood progenitors—are also driven by mutation of the protein calreticulin, a chaperone that prevents misfolded or incorrectly processed proteins from being transported to their destinations in the cell. In a March paper in *Blood*, researchers led by Ludwig investigator Stefan Constantinescu detailed how the mutation of calreticulin turns it into a rogue chaperone of TpoR. Stefan and his colleagues described the mechanism by which the aberrant calreticulin transports both normal and mutant TpoR to the cell-surface in states that would not ordinarily pass the cell's quality control processes. This rogue activity, they found, is required for the development of these two myeloproliferative disorders as it leads to activation of TpoR in the absence of its ligand.

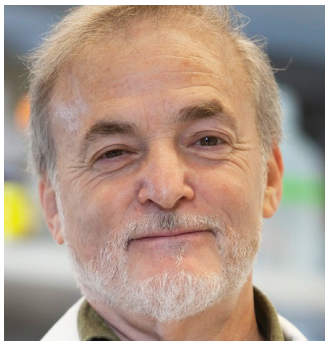
**Stefan Constantinescu**
**Ludwig Institute**

## BETTER BLADDER MONITOR

Bladder cancer, the tenth most common cancer worldwide, is often diagnosed late and tends to recur. Current methods of detection and patient surveillance are invasive and of limited sensitivity. A noninvasive test for early detection would help reduce costs and improve cure rates and the surveillance of survivors. In an April paper in *Cancer Discovery*, a team led by Ludwig Stanford researchers Ash Alizadeh and Maximilian Diehn reported a step in that direction—the development of uCAPP-Seq, a method for detecting bladder cancer DNA shed into urine. The researchers applied the test to 67 healthy adults and 118 patients with early-stage bladder cancer who had urine collected prior to treatment or during surveillance. Their test outperformed all existing diagnostics for the surveillance of bladder cancer patients, including the relatively insensitive but noninvasive cytology test and more accurate cystoscopy, in which the bladder is examined and tissue samples are taken for analysis. uCAPP-Seq detected all bladder cancers picked up by cytology and 82% that cytology missed. It could also detect bladder cancer 2.7 months earlier than could the current gold standard, cystoscopy.

Ash Alizadeh
Ludwig Stanford

Maximilian Diehn
Ludwig Stanford



Stephen Baylin
Ludwig Johns Hopkins

## DEADLY DOMAINS

Cancer cells have an uncanny ability to switch off the cellular mechanisms that prevent cancer initiation and growth. This is often accomplished by epigenetic modification, in which chemical tags are placed on DNA and its protein packaging to alter gene expression. One protein involved in this activity, UHRF1, guides to DNA the enzymes that add methyl groups to DNA across the genome to silence gene expression and maintains a profile of abnormal gene silencing in cancer. A team led in part by the Ludwig Professor Stephen Baylin at Johns Hopkins reported in an April paper in *Cancer Cell* its analysis of specific regions of UHRF1 that maintain the methylation of hundreds of cancer-fighting genes in colon cancer. Blocking those two domains—PHD and SRA—reversed the methylation of tumor-suppressing genes and revived their expression. In mice bearing human colon tumors, disrupting the two domains shrank tumors and slowed metastasis. Looking at human colon tumors from more than 300 patients, the researchers found that those with high levels of UHRF1 had lower levels of cancer-fighting genes. Clinical records of 150 patients showed that patients with high UHRF1 colon cancers suffered recurrence 20 months earlier and died two years earlier than those with normal UHRF1 expression. The researchers argue that specific targeting of PHD and SRA might offer a new approach to treating colon cancer.

## A TURNCOAT TARGET

Glioblastomas (GBM) are extremely complex and aggressive brain tumors that are difficult to treat and currently impossible to cure. Tumor growth can be slowed by radiation and chemotherapy following surgery, but the tumors quickly become resistant. In a March paper in *Cancer Cell*, a team led by Ludwig San Diego's Frank Furnari reported a novel mechanism by which such resistance develops. It involves a protein named PTEN, which is ordinarily a tumor suppressor. PTEN is frequently mutated in cancer and its loss has been linked to tumor growth and chemotherapy resistance in GBM. But it can also support tumor growth in certain circumstances. Frank and his colleagues found that phosphorylation of PTEN—the addition of a phosphate molecule to an amino acid of the protein, in this case tyrosine 240—promoted DNA repair in tumors, reversing the effects of therapeutic radiation. When the researchers blocked tyrosine 240 phosphorylation using inhibitors of the fibroblast growth factor receptor (FGFR) in mouse models of GBM, the cancer cells became sensitive to radiation, extending survival of the mice. This suggests FGFR antagonists might work as sensitizers to radiotherapy in GBM, and perhaps other cancers.

**Frank Furnari**
**Ludwig San Diego**

## HEAVY METAL THERAPY

A team led by Ludwig Harvard researcher Rakesh Jain explored how solid stress, or the "compressive and tensile mechanical forces" exerted by nodular brain tumors, affect people with brain cancers. Analyzing MRI scans of 64 glioblastoma patients' tumors and investigating tumors in mice, Rakesh and his team found that nodular tumors, which grow as a defined mass, cause more stress than infiltrative tumors that snake out into healthy tissue. Rakesh and his team reported in a March paper in *Nature Biomedical Engineering* that compression from nodular tumors decreases blood flow in nearby blood vessels, damaging surrounding neural tissue—findings that they confirmed in analyses of two other groups of patients, including women with nodular breast cancer metastases. The researchers treated mice with four drugs known to have neuroprotective effects to examine whether the damage from solid stress could be reversed. Only one, lithium, a drug that has long been used to treat bipolar disorder, appeared to reduce neuronal death. Lithium-treated mice also performed significantly better in movement and motor coordination tests than did untreated animals. A brief video summarizing the findings can be viewed here.

**Rakesh Jain**
**Ludwig Harvard**

## GENTLE TAPS

Chemical modifications to DNA—or "epigenetic" marks—help control gene expression, and their aberrant distribution across the genome contributes to cancer progression and resistance to therapy. Two of the most common modifications of this sort involve the addition of methyl and hydroxymethyl groups to the DNA base cytosine to create 5mC and 5hmC. Molecular biologists have long relied on a method known as bisulfite sequencing to detect the modifications. Unfortunately, the method destroys as much as 99% of the sequenced DNA, which can pose a serious practical challenge when the sample in question is in precious supply. Ludwig Oxford's Chunxiao Song and Benjamin Schuster-Böckler have developed a new method to detect the two modifications that is half as expensive to sequence, twice as fast to analyze and does much less damage to DNA than bisulfite sequencing, leaving more of the sample intact for additional structural and mutational analyses. The new method—named TET-assisted pyridine borane sequencing, or TAPS—was described in an April issue of *Nature Biotechnology*. Chunxiao, Benjamin and their colleagues are now refining TAPS to enable the analysis of rare fragments of DNA shed by tumors into the bloodstream, with the aim of developing minimally invasive cancer diagnostics.

Chunxiao Song
Ludwig Oxford

Benjamin Schuster-Böckler
Ludwig Oxford

Colin Goding
Ludwig Oxford

## RED ALERT

Redheads have a 10- to 100-fold greater risk of melanoma and, even though they make up only 1-2% of the population, account for 16% of those with the skin cancer. Red hair color is caused by variations in the Melanocortin 1 Receptor (MC1R), a cell-surface signaling protein crucial for pigmentation in humans. The variations result in the production of a pigment that limits the skin's defense against UV radiation, the leading cause of melanoma. MC1R signaling is dependent on a chemical modification made to the receptor known as palmitoylation, so it has been hypothesized that increasing MC1R-palmitoylation might be one way to help protect people with red hair from the skin cancer. Just how that might be done has, however, remained a mystery. In a February paper in *Nature Communications*, Ludwig Oxford's Colin Goding, who has a personal interest in the topic as he is a compound MC1R heterozygote, and colleagues from Boston University School of Medicine and Shandong Normal University reported a possible approach to maintaining palmitoylation of MC1R. They identified an enzyme named APT2 as the agent that removes the palmitoyl group from MC1R and showed that its inhibition can remedy the signaling defects of variant MC1Rs and suppress the emergence of melanoma in a mouse model in response to UV irradiation. APT2 inhibition could thus be a means to reducing melanoma risk in redheads and others.

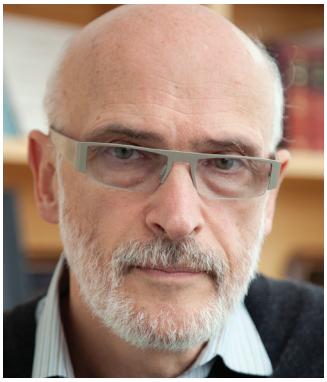
## TUMOR THERMOSTAT

Some 40% of melanoma patients fail to benefit from even a combination of anti-PD1 and anti-CTLA4 checkpoint blockade. This is often because their "cold" tumors are insufficiently infiltrated by tumor targeting T cells. Ludwig Lausanne's Ping-Chih Ho and colleagues reported in a February paper in *Nature Immunology* a cellular mechanism that heats up "cold" melanoma tumors and an existing drug that can induce that effect in a mouse model of the cancer. Ping-Chih and his team compared hot and cold melanoma tumors and identified a metabolic protein, UCP2, that is highly expressed in the former. They then engrafted melanoma tumors that could be prompted to express high levels of UCP2 into mice and showed that UCP2 expression drew killer T cells and conventional type 1 dendritic cells (cDC1), which activate killer T cells, into the tumors. Mice engineered to lack cDC1 cells did not show this response. In cold melanoma tumors, inducing UCP2 expression before treatment with an anti-PD1 antibody elicited robust anti-tumor immune responses that significantly extended survival of mice. The researchers showed that a diabetes drug known to induce UCP2 expression, rosiglitazone, could also sensitize cold tumors to checkpoint blockade and extend the survival of mice. Notably, the drug induced UCP2 expression in cultures of melanoma cells obtained from patients as well.

**Ping-Chih Ho**
**Ludwig Lausanne**

## IDENTITY SUPPORT

Regulatory T cells (Treg cells) are immune cells that play a critical role in many biological processes, from suppressing inflammation and deadly autoimmunity to helping tumors evade immune attack. Their identity is established by a master regulator of gene expression known as Foxp3. But Treg cells also express a related transcription factor: Foxp1. In a February paper in *Nature Immunology*, a team led by Ludwig MSK Director Alexander Rudensky reported that a large number of Foxp3-bound genomic sites in Treg cells are also occupied by Foxp1 in both Treg cells and conventional T cells, and that FoxP1 supports FoxP3 binding to those sites. A deficiency of FoxP1 resulted in the lowered expression of a variety of genes essential to Tregs and impaired their function. Enhanced signaling from an immune factor key to Treg activity known as interleukin-2 (IL-2) partly reversed these defects. Alexander and his colleagues argue, based on their findings, that Foxp1 serves an essential and non-redundant function in Treg cells by enforcing Foxp3-mediated regulation of gene expression and enabling efficient IL-2 signaling in these cells.

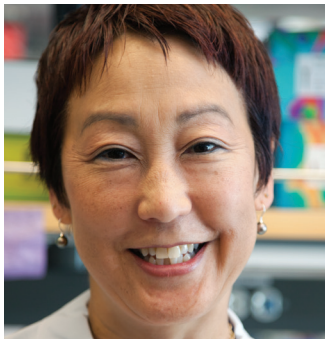


**Alexander Rudensky**
**Ludwig MSK**

## DIETARY RESCUE

A study led by Ludwig Lausanne's Nicola Vannini and Olaia Naveiras of the Ecole Polytechnique Fédérale de Lausanne has found that a common dietary supplement dramatically boosts the production of blood cells stemming from hematopoietic stem cells (HSCs) in mice. If applicable to humans, the finding could help reduce the toxicity of stem cell-based therapies for leukemia and aggressive lymphomas that involve the destruction of HSCs and their replacement with healthy ones. About a quarter of patients die from such treatments because their immune cells are not replenished quickly enough. Nicola and his colleagues reported in a March issue of *Cell Stem Cell* that this happens because the effort to replace blood causes mitochondrial stress in HSCs that significantly ages the organelles, best known as power generators for the cell. Exposing human and mouse HSCs to nicotinamide riboside, an analogue of vitamin B3, boosts their function, ability to replace stressed-out mitochondria and multiply. After mice were exposed to intense irradiation that virtually eliminated their ability to make blood and transplanted with a limited number of hematopoietic stem cells, the supplement accelerated blood recovery and improved their survival by 80%. In immunodeficient mice, it increased the production of human blood and immune cells. This is the first study to suggest that dietary supplementation with nicotinamide riboside might amp up blood-recovery after chemo- or radiotherapy.

Nicola Vannini
Ludwig Lausanne



Judith Shizuru
Ludwig Stanford

## A GENTLER FIX

Researchers led by Ludwig Stanford researcher Judith Shizuru reported in a *Blood* paper published online in February that an antibody can gently eliminate diseased and healthy hematopoietic, or blood-forming, stem cells in the bone marrow to prepare for the transplantation of healthy stem cells. Their approach could circumvent the need for potentially life-threatening chemotherapy or radiation to prepare patients for such transplants and greatly expand the pool of eligible patients. The antibody targets CD117, a protein on hematopoietic stem cells (HSCs). Judith and her colleagues applied it in a mouse model of myelodysplastic syndrome (MDS), blood disorders stemming from aberrant HSCs that cause an abnormal proliferation of various blood cells. Anti-CD117 antibodies gently removed both MDS and normal HSCs and eased their replacement with healthy HSCs, restoring the normal production of blood cells. The findings have implications for the treatment of a range of autoimmune, developmental and malignant disorders. This and an earlier study in *Science Translational Medicine* have led to regulatory approval for two blood stem cell transplant clinical trials for different diseases. The first, currently underway, is testing the antibody in children with severe combined immunodeficiency—popularly known as "bubble boy disease" because sufferers lack a functional immune response and must be sequestered from all pathogens. Early results are promising. The second trial will treat elderly adults with MDS to determine if a gentler cure can be achieved.

**Top left:** From left, Ece Auffarth, Alessandro Peschechera (alum) and Rachel Reinhardt of the Ludwig Institute; Benjamin Schuster-Böckler of Ludwig Oxford; and Bob Strausberg of the Ludwig institute.

**Top center:** Gerd Ritter, left, of the Ludwig Institute; Robert Weinberg of Ludwig MIT; and Robert Schreiber of the Washington University School of Medicine

**Top right:** Benoît Van den Eynde, left, and Jonathan C.A. Skipper of the Ludwig Institute.

**Left:** Joan Brugge, left, co-director of Ludwig Harvard; and Xin Lu, director of Ludwig Oxford.

## MEETING UP IN ATLANTA

More than 75 Ludwig-affiliated scientists and staff attended the 110th AACR Annual Meeting in Atlanta, Georgia, home to CNN, Coca-Cola and excellent barbecue. They shared their data and insights on everything from the tumor microenvironment to combination immunotherapies to liquid biopsies for early cancer detection to precision cancer medicine. Many also attended Ludwig's traditional cocktail hour and reception, where they swapped stories with old colleagues and met others for the first time. Here are a few pictures from the meeting and the reception. Click here for a more comprehensive account of Ludwig's participation in the conference.

# RAVI MAJETI

## LUDWIG STANFORD

**What role do stem cells play in cancer development?**

One of the most important properties of stem cells is self-renewal—the process by which they divide to make more stem cells and perpetuate the stem cell pool throughout a life. Their role in cancer is a complicated and sometimes controversial issue. Not all cancer cells are the same and within a malignant tumor or among the circulating cancerous cells of leukemia, for example, there can be cells of different function and potential. The stem cell theory of cancer proposes that among all cancerous cells, a subset act as stem cells that reproduce themselves and sustain the cancer, much like normal stem cells normally renew and sustain our organs and tissues.

**What are stem cells and why are they so important?**

There are two categories of stem cells. The first are adult stem cells that reside in individual tissues or organs. The blood, or hematopoietic, stem cell is perhaps the best understood and most accessible. It can differentiate into all types of blood cells—red cells, white cells, platelets—and produce all cell lineages within the adult blood system. They are also one of the few stem cell types with a long history of clinical application, in the form of bone marrow transplantation. The second type are what are called pluripotent stem cells.

These include embryonic stem cells, which are derived from human embryos and induced pluripotent stem cells, a type of stem cell developed over the last decade as a major advance in the field of stem cell biology. They have enormous regenerative medicine potential because they have the ability to give rise to all the cell types in the body and can be cultured and expanded in the laboratory. But the translation of the pluripotent stem cells into clinical applications is really in its infancy and there are many basic issues that still have to be solved to bring those approaches into the clinic.

**Your lab has two goals. You focus on the therapeutic targeting of leukemia stem cells, but you also focus on normal human hematopoiesis. How are these two goals related?**

The overarching goal of our research is to develop new therapeutic approaches to treating leukemia and thereby treating leukemia stem cells. But in order to do that we have to know what are the vulnerabilities specific to the leukemia stem cells and, in particular, how are they different from normal blood stem cells. So understanding normal blood stem cell biology and development is critical to identifying new approaches to treating leukemia.

**What's missing from a technical perspective that will help move the field of stem cell research forward?**

We can put that into two bins: the scientific questions and the regenerative medicine translational applications. What's missing in both is not one major element but a number of smaller issues that need to be solved in order to advance the field. One critical scientific question we need to address is how do we model complex multi-factorial diseases, such as neurological or psychiatric diseases, using stem cell biology, whether they be *ex vivo* in the laboratory or in animal models. Modeling these complex diseases using stem cells is really key to moving the field forward. Translational regenerative medicine applications, on the other hand, include the ability to expand cells, scale the process and manufacture them under GMP (Good Manufacturing Process) conditions in order to safely

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administer them to individuals. Some of the challenges we face here are how to integrate cells that are engineered outside the body into an existing organ and get them to function cooperatively with the other cells that are already present and how to make an organ that could be transplanted into an individual who needs regenerative therapy.

**Does treating patients have a significant influence on your work as a scientist?**
I have trained extensively in the treatment of leukemia patients both with chemotherapy and bone marrow transplantation. Those experiences have really helped guide my research in many ways. Right now, I don't take care of leukemia patients, but I'm actively participating in our clinical programs. This has an enormous impact on my research as it has allowed me to envision the big picture by asking: What are the most clinically relevant questions? And, maybe more importantly, what are not clinically relevant questions? Resources and time

are limited, so you have to make those tough choices.

**One of your many leadership roles at Stanford has been as co-director of the Translational Investigator Pathway (TIP) for the Internal Medicine Residency Program. What excites you about this program?**

The Stanford University School of Medicine has a long, outstanding tradition of training physician-scientists who have gone on to become leaders in academic and translational medicine. I have a deep passion for mentoring physician-scientist trainees and the TIP program is an invaluable and important part of my activities here. Everyone involved seeks to link our trainees with potential mentors so they can identify areas they'd like to pursue during their research training years. Physician-scientists play a unique and critical role in medical research and a lot of translational innovation comes from this group. But they are a dying breed across the U.S. and the number of medical students interested in pursuing careers as physician-scientists has declined over the past 20 years. For the sake of medical innovation, it's imperative we recruit and train a new crop of them. But it's a challenge because physician-scientists often drop off the pathway towards becoming translational researchers because they don't see very many role models or even peer models around them. Providing longitudinal mentorship and even trainee-to-trainee mentorship is invaluable. Ultimately, it's really about building the next generation of translational investigators who will keep advancing the field.

**Juggling your work as a physician-scientist, you must have met challenges along the way. Do you have any advice for junior scientists facing challenges?**

I have the great privilege of mentoring lots of junior scientists and junior physician-scientists. There will always be personal and professional challenges in any career, and my advice is to focus and commit yourself to doing what is most interesting and important to you. Take on those activities that will sustain you intellectually for your whole career. Too often we focus on the short-term, which is often based on real-world issues like family and finances. But it's really important to think about the big picture in the arc of a career. At the beginning of their training, physician-scientists are presented with a number of amazing opportunities—research, teaching, clinical practice. It's okay to prioritize them in different ways. They just don't need to be prioritized equally. And I think it's very difficult to pursue them all at the highest level. That's a trap that some junior scientists fall into. Finding the right mentor—or I should say mentors—is key because you need multiple types of mentorship. My final piece of advice is: life intervenes. That's OK. Sometimes people feel that they've lost out or they're not succeeding because they can't handle everything all at once. No one can.

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**How do you think we can reach out to the younger generation and encourage more young, bright minds into science?**
Outreach is key, especially in the middle schools and high schools. That’s where you can really hook young people on the excitement of science and scientific inquiry. They need role models to give them real-world examples of what a career in science is really like. Otherwise, the only examples they’re exposed to are from the movies or from television. As scientists, we need to be able to convey complex scientific projects and principles in a way that young people can understand them. That means we need to get out of our offices and ivory towers and speak clearly and coherently about what we are doing, why it’s important, what the problems are that we are trying to solve and how science is the pathway to solving them.

**Does Stanford have any youth science programs?**
We have a number of great programs for middle school and high school students. Some are targeted toward underrepresented minority students and others toward any student who applies. We have short-term weekend opportunities that expose kids to biomedicine and biomedical sciences and summer programs where high school students can spend eight weeks in a laboratory learning some of the basics of scientific research along with a classroom-based curriculum. The problem is that there are way more students interested in participating than we can host. We should never turn away a student who wants to participate in one of our programs. Everyone should be given an opportunity to test-drive a career in science.

**Why do you think Acute Myeloid Leukemia (AML) is the cancer with the strongest evidence for the critical involvement of cancer stem cells?**
The biology of AML is different than that of other tumors because the leukemia cells are liquid and circulating so they are less dependent on interactions with other cells. The cancers that occur in solid organs are more dependent on their microenvironment, which is a biologic distinction that also contributes to different interpretations of the

cancer stem cell model. The stem cell hierarchy and blood development has been clearly elucidated and the assays for normal blood stem cells have been applied to the investigation of AML. And it's the presence of those assays that has propelled AML to the forefront of the cancer stem cell research field. I also think that AML is really just hijacking aspects of this normal blood stem cell hierarchy and so it's been easier to ascertain the experimental evidence for the critical involvement of cancer stem cells in AML. Since the blood system is liquid and moves around the body, it's not set in a physical location or in a physical structure. It's unlike a solid organ where there are multiple cellular subtypes that are physically linked, which makes the experimental process of isolating individual cells and studying them potentially a confounder in the interpretation of the experiments. This means that the cells in contact with other cells and components in an organ may have different properties and behave differently after you have dissected the tissue, digested it and prepared single cells for study.

**You have a number of academic and administrative appointments in addition to your clinical work. What do you do to relax and have fun?**

I've always made it a high priority to spend time with my family. I have two sons who are in high school but over the ten-year arc of my career, they went from little boys to young men. We like to travel and my wife has been the key in insisting that I unplug when we go on vacation instead of sitting and looking at my computer in the evenings when we come back to the hotel. I'm also a basketball junkie. I've played basketball all my life. I've coached my sons' basketball teams, I watch basketball, I talk about basketball, it's hard to find somebody who's more into basketball than me. When I was in medical and graduate school, before I met my wife and had kids, I played every day. I still play full court basketball once a week even though I probably should stop because I'm getting too old for all the contact.

**And your favorite team?**

Golden State Warriors— I'm a Bay Area guy through and through. How can you not love Steph Curry? Come on now!

# What is the biggest challenge facing cancer researchers and oncologists today?

The speed and efficiency with which physicians match cancer treatment to each patient's cancer-genome suffers from an inability to quickly cross reference treatment efficacy with genetic markers. Rapidly screening a limited number of cancer cells against thousands of existing drugs to determine the most effective treatment would help physicians provide personalized medicine in a timely way.

TIAN YI ZHANG
Ludwig Stanford

For the preclinical scientist interested in understanding how cancers arise, there is the challenge of trying to understand the extraordinarily complex intracellular signaling network—i.e., the "wiring diagram" of the complex network of interacting signal-processing proteins—that governs the decisions of a cell about whether or not it should divide, grow or die.

For the clinical oncologist intent on treating existing cancers, there is the daunting problem of anticipating and/or responding to the plasticity of cancer cell populations and their proclivity to spawn treatment-resistant variant subpopulations that grow out and yield clinical relapse after a period of responsiveness to initially effective therapeutics.

ROBERT WEINBERG
Ludwig MIT

Due to cancer's complexity, there are no perfect experimental cancer models, which hampers a thorough understanding of disease progression and resistance to different therapies. We need better models to predict human responses to new therapies to maximize the success rate of expensive and lengthy clinical trials.

CAROL LEUNG
Ludwig Oxford

# Required reading

## Ludwig Brussels

**Blood 2019 March 22 [Epub ahead of print]**
**Calreticulin mutants as oncogenic rogue chaperones for TpoR and traffic-defective pathogenic TpoR mutants.**

Pecquet C, Chachoua I, Roy A, Balligand T, Vertenoeil G, Leroy E, Albu RI, Defour JP, Nivarthi H, Hug E, Xu E, Ould-Amer Y, Mouton C, Colau D, Vertommen D, Shwe MM, Marty C, Plo I, Vainchenker W, Kralovics R, Constantinescu SN.

**Journal of Immunology 2019 January 15**
**The vacuolar pathway of long peptide cross-presentation can be TAP dependent.**

Ma W, Stroobant V, Heirman C, Sun Z, Thielemans K, Mulder A, van der Bruggen P, Van den Eynde BJ.

## Ludwig Chicago

**Nature Communications 2019 Apr 23**
**Immunostimulatory nanomedicines synergize with checkpoint blockade immunotherapy to eradicate colorectal tumors.**

Duan X, Chan C, Han W, Guo N, Weichselbaum RR, Lin W.

## Ludwig Harvard

**Cell Research 2019 June**
**Vulnerabilities in mIDH2 AML confer sensitivity to APL-like targeted combination therapy.**

Mugoni V, Panella R, Cheloni G, Chen M, Pozdnyakova O, Stroopinsky D, Guarnerio J, Monteleone E, Lee JD, Mendez L, Menon AV, Aster JC, Lane AA, Stone RM, Galinsky I, Zamora JC, Lo-Coco F, Bhasin MK, Avigan D, Longo L, Clohessy JG, Pandolfi PP.

**Nature Genetics 2019 May**
**Detecting the mutational signature of homologous recombination deficiency in clinical samples.**

Gulhan DC, Lee JJ, Melloni GEM, Cortés-Ciriano I, Park PJ.

**Proceedings of the National Academy of Sciences USA 2019 May 28**
**Reprogramming the microenvironment with tumor-selective angiotensin blockers enhances cancer immunotherapy.**

Chauhan VP, Chen IX, Tong R, Ng MR, Martin JD, Naxerova K, Wu MW, Huang P, Boucher Y, Kohane DS, Langer R, Jain RK.

**Nature Metabolism 2019 April 8**
**miR-147b-mediated TCA cycle dysfunction and pseudohypoxia initiate drug tolerance to EGFR inhibitors in lung adenocarcinoma.**

Zhang WC, Wells JM, Chow K-H, Huang H, Yuan M, Saxena T, Melnick MA. Politi K, Asara JM, Costa DB, Bult CJ, Slack FJ.

**Nature Biomedical Engineering 2019 March**
**Solid stress in brain tumours causes neuronal loss and neurological dysfunction and can be reversed by lithium.**

Seano G, Nia HT, Emblem KE, Datta M, Ren J, Krishnan S, Kloepper J, Pinho MC, Ho WW, Ghosh M, Askoxylakis V, Ferraro GB, Riedemann L, Gerstner ER, Batchelor TT, Wen PY, Lin NU, Grodzinsky AJ, Fukumura D, Huang P, Baish JW, Padera TP, Munn LL, Jain RK.

## Ludwig Johns Hopkins

**JAMA Oncology 2019 May 9 [Epub ahead of print]**
**Prognostic potential of circulating tumor DNA measurement in postoperative surveillance of nonmetastatic colorectal cancer.**

Wang Y, Li L, Cohen JD, Kinde I, Ptak J, Popoli M, Schaefer J, Silliman N, Dobbyn L, Tie J, Gibbs P, Tomasetti C, Kinzler KW, Papadopoulos N, Vogelstein B, Olsson L.

**Cancer Cell 2019 April 15**
**Defining UHRF1 domains that support maintenance of human colon cancer DNA methylation and oncogenic properties.**

Kong X, Chen J, Xie W, Brown SM, Cai Y, Wu K, Fan D, Nie Y, Yegnasubramanian S, Tiedemann RL, Tao Y, Chiu Yen RW, Topper MJ, Zahnow CA, Easwaran H, Rothbart SB, Xia L, Baylin SB.

## Ludwig Lausanne

**Cell Stem Cell 2019 March 7**
**The NAD-booster nicotinamide riboside potently stimulates hematopoiesis through increased mitochondrial clearance.**

Vannini N, Campos V, Girotra M, Trachsel V, Rojas-Sutterlin S, Tratwal J, Ragusa S, Stefanidis E, Ryu D, Rainer PY, Nikitin G, Giger S, Li TY, Semilietof A, Oggier A, Yersin Y, Tauzin L, Pirinen E, Cheng WC, Ratajczak J, Canto C, Ehrbar M, Sizzano F, Petrova TV, Vanhecke D, Zhang L, Romero P, Nahimana A, Cherix S, Duchosal MA, Ho PC, Deplancke B, Coukos G, Auwerx J, Lutolf MP, Naveiras O.

**Nature Immunology 2019 February**
**Uncoupling protein 2 reprograms the tumor microenvironment to support the anti-tumor immune cycle.**

Cheng WC, Tsui YC, Ragusa S, Koelzer VH, Mina M, Franco F, Läubli H, Tschumi B, Speiser D, Romero P, Zippelius A, Petrova TV, Mertz K, Ciriello G, Ho PC.

## Ludwig MIT

**Proceedings of the National Academy of Sciences USA 2019 May 8 [Epub ahead of print]**
**Noninvasive imaging of tumor progression, metastasis, and fibrosis using a nanobody targeting the extracellular matrix.**

Jailkhani N, Ingram JR, Rashidian M, Rickelt S, Tian C, Mak H, Jiang Z, Ploegh HL, Hynes RO.

## Ludwig MSK

**Nature Immunology 2019 February**
**Transcription factor Foxp1 regulates Foxp3 chromatin binding and coordinates regulatory T cell function.**

Konopacki C, Pritykin Y, Rubtsov Y, Leslie CS, Rudensky AY.

## Ludwig Oxford

**Autophagy 2019 May**
**Autophagy inhibition specifically promotes epithelial-mesenchymal transition and invasion in RAS-mutated cancer cells.**

Wang Y, Xiong H, Liu D, Hill C, Ertay A, Li J, Zou Y, Miller P, White E, Downward J, Goldin RD, Yuan X, Lu X.

# Required reading

**Nature Biotechnology 2019 April**
**Bisulfite-free direct detection of 5-methylcytosine and 5-hydroxymethylcytosine at base resolution.**

Liu Y, Siejka-Zielińska P, Velikova G, Bi Y, Yuan F, Tomkova M, Bai C, Chen L, Schuster-Böckler B, Song CX.

**Nature Communications 2019 February 20**
**Targeting MC1R depalmitoylation to prevent melanomagenesis in redheads.**

Chen S, Han C, Miao X, Li X, Yin C, Zou J, Liu M, Li S, Stawski L, Zhu B, Shi Q, Xu ZX, Li C, Goding CR, Zhou J, Cui R.

## Ludwig San Diego

**Nature 2019 May**
**NAD metabolic dependency in cancer is shaped by gene amplification and enhancer remodelling.**

Chowdhry S, Zanca C, Rajkumar U, Koga T, Diao Y, Raviram R, Liu F, Turner K, Yang H, Brunk E, Bi J, Furnari F, Bafna V, Ren B, Mischel PS.

**Cancer Cell 2019 March 18**
**Inhibition of nuclear PTEN tyrosine phosphorylation enhances glioma radiation sensitivity through attenuated DNA repair.**

Ma J, Benitez JA, Li J, Miki S, Ponte de Albuquerque C, Galatro T, Orellana L, Zanca C, Reed R, Boyer A, Koga T, Varki NM, Fenton TR, Nagahashi Marie SK, Lindahl E, Gahman TC, Shiau AK, Zhou H, DeGroot J, Sulman EP, Cavenee WK, Kolodner RD, Chen CC, Furnari FB.

## Ludwig Stanford

**Blood 2019 May 9**
**Anti-CD117 antibody depletes normal and myelodysplastic syndrome human hematopoietic stem cells in xenografted mice.**

Pang WW, Czechowicz A, Logan AC, Bhardwaj R, Poyser J, Park CY, Weissman IL, Shizuru JA.

**Nature Biotechnology 2019 May 6 [Epub ahead of print]**
**Determining cell type abundance and expression from bulk tissues with digital cytometry.**

Newman AM, Steen CB, Liu CL, Gentles AJ, Chaudhuri AA, Scherer F, Khodadoust MS, Esfahani MS, Luca BA, Steiner D, Diehn M, Alizadeh AA.

**Cancer Discovery 2019 April**
**Detection and surveillance of bladder cancer using urine tumor DNA.**

Dudley JC, Schroers-Martin J, Lazzareschi DV, Shi WY, Chen SB, Esfahani MS, Trivedi D, Chabon JJ, Chaudhuri AA, Stehr H, Liu CL, Lim H, Costa HA, Nabet BY, Sin MLY, Liao JC, Alizadeh AA, Diehn M.

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