

# LUDWIG LINK

OCTOBER 2018

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LUDWIG  
CANCER  
RESEARCH

LIFE-CHANGING SCIENCE



## LETTER



Ludwig's scientists have never wanted for creativity, and there's ample evidence of that in our latest issue of Ludwig Link. Read on and you'll learn about how our researchers are developing a liquid biopsy to predict the outcome of cancer therapy,

applying nanotechnology to pierce the armor of the pancreatic tumor, devising probes to track activated T cells in patients receiving immunotherapy, exploring the interplay of immune cells and commensal microbes in the gut—and much, much more.

Big data is already a big deal in cancer research and care, and the Q&A in this issue touches on a Ludwig program in this exciting arena. Ludwig Harvard's Peter Sorger, who is leading the Ludwig Cancer Atlas project, discusses how he and collaborating researchers are developing microscopes to generate high-dimensional maps of tumors and adding artificial intelligence to the mix to advance cancer diagnostics and therapy.

And speaking of big things, in the era of personalized medicine, are smaller, more targeted clinical trials set to replace large, randomized studies in cancer drug development? We asked Ludwig researchers to weigh in on the matter. Their answers are on page 20.

Finally, we'd like to give a big welcome to Ludwig's newest board member, Nancy Davidson, who joined our community in August. She is briefly profiled on page 4.

Wishing you all a happy fall!

Rachel Reinhardt  
Vice President for Communications

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**On the cover:** Sudhir Chowdhry, left, and Paul Mischel, Ludwig San Diego



Nancy E. Davidson  
Ludwig Institute Board  
of Directors

## NEW TO THE LUDWIG BOARD

Nancy E. Davidson was appointed to the Ludwig Institute Board of Directors in August. Nancy is a renowned physician-scientist noted for her work on breast cancer, but she wears many hats: senior vice president and director of the Clinical Research Division and endowed chair for Breast Cancer Research at the Fred Hutchinson Cancer Research Center, head of the Division of Medical Oncology in the Department of Medicine at the University of Washington and executive director and president of Seattle Cancer Care Alliance. This past June she received the Allen S. Lichter Visionary Leader Award, which honors those who have significantly

changed the field of oncology or made outstanding contributions to further the missions of the American Society of Clinical Oncology (ASCO), CancerLinQ LLC, or Conquer Cancer. Nancy has also served as president of both the American Association for Cancer Research (AACR) and ASCO. She received her medical degree from Harvard Medical School and her bachelor's degree from Wellesley College. We are delighted to have Nancy on board and are sure her guidance, given her breadth of experience in both basic and clinical research, will serve Ludwig well.

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## News roundup



Richard Kolodner  
Ludwig San Diego

## EDITORIAL DOMAIN

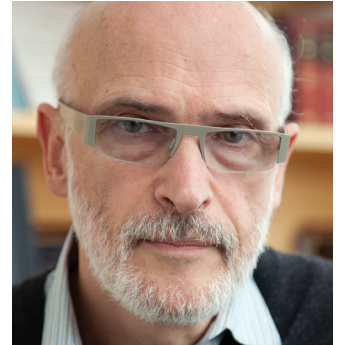
Every time a cell divides, it must copy and bequeath to its daughter cells a replica of its DNA sequence, totaling some 3 billion base pairs. But nothing is perfect and though cells employ an arsenal of editing tools to correct errors in DNA replication, some mistakes go unrepaired, seeding mutations that can cause or predispose people to cancer. In an August [paper](#) in *Nature Structural & Molecular Biology*, Ludwig San Diego Director Richard Kolodner and his colleagues report a hitherto unknown protein domain—a conserved, functional element of protein structure—that plays an important role

in repairing mismatched DNA bases. The domain, which Richard and colleagues discovered in yeast and named SHIP, was initially identified at the tail end of Exo1, an enzyme that snips DNA to initiate repair. The researchers identified two other yeast proteins that carry the SHIP domain, as well as three candidate human proteins that appear to do so. Their studies support a model for mismatch repair in which Exo1 is tethered to a protein complex that recognizes mispaired bases and stimulates the excision of DNA strands in preparation for repair.

## ALTERED STATES

A malignant tumor is a complex ecosystem. It includes fat, immune and other types of cells that provide support, plumbing and nourishment to cancer cells. A [study](#) led by Ludwig MSK Director Alexander Rudensky and Dana Pe'er, Chair of the Computational and Systems Biology Program, Sloan-Kettering Institute, published in an August issue of *Cell* explored the diversity of immune cells in tumors. The researchers performed single-cell sequencing on more than 45,000 immune cells from eight tumors as well as 27,000 additional immune cells and applied a computational tool to characterize each population. Their results reveal that there isn't any clear-

cut difference between the types of immune cells found in tumors and normal tissues. What distinguishes those found in tumors from their healthy counterparts is the vastly greater diversity of cellular states—or phenotypes—in which they exist. This may help explain why individual tumors respond so differently to the same immunotherapy and, someday, aid in the better personalization of such treatments. The findings also have implications for how tumor-infiltrating immune cells are characterized. (For more on Alexander's fascinating life, career and contributions to immunology, see our profile in the [2018 Research Highlights](#) report.)

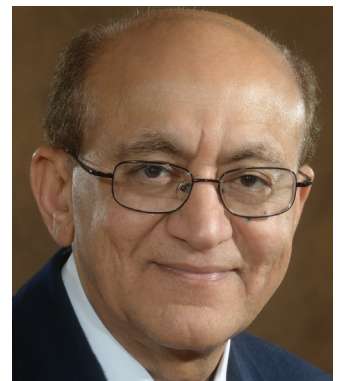


Alexander Rudensky  
Ludwig MSK

## POSITIVE VIBRATION

Brain cancers are difficult to treat in part because most therapeutic drugs are shut out by the protective blood-brain barrier. In a [paper](#) published online in August in the *Proceedings of the National Academy of Sciences*, a team of researchers led by Ludwig Harvard's Rakesh Jain describes the mechanisms underlying an approach to improve anti-cancer drug delivery to the brain. Known as focused ultrasound, the technology trains multiple ultrasound beams on a single point, where drug-filled lipid bubbles that vibrate under the pulse can traverse the barrier. The researchers used advanced microscopy and mathematical modeling

to analyze and quantify the effects of focused ultrasound on cell transport and tissues. They discovered that focused ultrasound increases the permeability of epithelial cells that line tumor blood vessels and boosts intracellular trafficking dramatically: It improved passage of the chemotherapy doxorubicin and the targeted drug T-DM1 across the cell membrane more than 20-fold and enhanced drug delivery throughout tumors in a mouse model of breast cancer brain metastasis. The results should help optimize the use of focused ultrasound for brain cancer therapy.



Rakesh Jain  
Ludwig Harvard



Ash Alizadeh

Ludwig Stanford



Maximilian Diehn

Ludwig Stanford

## EARLY WARNING

A Ludwig Stanford team led by Maximilian Diehn and Ash Alizadeh has devised a blood test that can determine in as little as three weeks whether patients with diffuse large B cell lymphoma will respond to standard therapy or require more aggressive treatment. About a third of patients with this blood cancer do not respond to the usual treatment but their doctors typically must wait months to discover it has failed, delaying the potentially lifesaving initiation of alternative treatments. For the [study](#), published in August in the *Journal of Clinical Oncology*, the researchers tracked levels of circulating tumor DNA (ctDNA)—which is shed by dying cancer cells—in 217 patients. Using a technique called Cancer Personalized Profiling by Deep Sequencing, or CAPP-Seq, they were able to detect ctDNA in 98% of patients prior to treatment. They found that patients whose ctDNA levels dropped hundredfold after the first round of chemotherapy or three-hundredfold following the second were far more likely to live two years or more without cancer recurrence than those whose ctDNA levels declined slowly. The researchers are hopeful that their approach will work for other types of cancer as well.

## BASICALLY A REVIVAL

Baking soda, an inexpensive kitchen staple, could give a boost to cancer therapy. A team of researchers led by Ludwig Scientific Director Chi Van Dang reported in a June [issue](#) of *Cell* a novel mechanism by which cells enter a state of metabolic latency as tissues starved of oxygen become increasingly acidic. Such dormancy makes cancer cells less susceptible to chemotherapy, and latent cells can seed relapses after therapy. Chi's team found that baking soda, a base that neutralizes acids, tricks such cells into reviving their metabolic activity—which should render them susceptible to chemotherapy. Turns out that acidity

inactivates a key regulator of metabolic activity, mTORC1, by propelling the protein complex to the periphery of the cell, away from a protein that helps switch it on. Neutralizing the acidity sends mTOR back to the nuclear locale of its activator, where it blinks back on—a phenomenon confirmed in tumors taken from mice that had ingested baking soda in water. Chi's team also found that T cell activation, which is essential to most immunotherapies, is similarly compromised in acidic conditions. They are now examining how acidity affects immunotherapy and further exploring its effects on cells.



Chi Van Dang  
Ludwig Institute



Sangeeta Bhatia  
Ludwig MIT

## BREACHING BARRIERS

Pancreatic ductal adenocarcinoma (PDAC), with a 5-year survival rate of 8.5%, is as deadly as it is in part because the dense, fibrous tissue that encases these tumors, called desmoplastic stroma, is virtually impermeable to drugs. Further, while some genetic targets of the cancer have been defined, they are considered 'undruggable'—the existing arsenal of delivery tools are largely incapable of hitting the few that are known. A team led by Ludwig MIT's Sangeeta Bhatia tested whether these therapeutic barriers could be overcome with RNA drugs and a nanotechnology-based delivery system. The researchers constructed

nanocomplexes (TPNs) made by combining a cell-penetrating peptide with the cyclic peptide iRGD, which is known to traverse the stromal barrier. They then packaged within it a small interfering RNA (siRNA) that targets the cancer-driving gene Kras. Sangeeta and her colleagues [reported](#) in *Molecular Cancer Therapeutics* in August that TPNs carrying this siRNA crossed the desmoplastic stroma and significantly delayed tumor growth in mouse models of PDAC. Notably, the modular design of the TPNs should allow for the targeting of other PDAC oncogenes as well.

## OUT OF CIRCULATION

Cancer cells shed by tumors that circulate in the bloodstream are rich with information. They have the potential to help refine the diagnosis of cancer, select appropriate drugs and monitor the efficacy of chosen therapies. Trouble is, these circulating tumor cells (CTCs) are rare, hard to detect and harder still to collect in useful quantities. In a [paper](#) published in July in the journal *Nature Biomedical Engineering*, Ludwig Stanford's Sanjiv Sam Gambhir and his colleagues report an elegant method for their collection that involves threading a thin patented magnetic wire a couple of inches long into a vein. The wire captures nanoparticles coated with antibodies that specifically tack onto the CTCs in the blood. Tested in a porcine model, the system collected between 500 and 5000 as many CTCs as a commercially available cell collector. It could also detect useful CTCs in less than 10 seconds; in 20 minutes it collected as many as you might ordinarily find in 80 tubes of blood. This approach can be generalized to detect other biomarkers in the blood, including circulating tumor DNA, and this is being studied. Sam and his team are conducting toxicity studies on the nanoparticles in mice and seeing if they can further ease the translation of their system by tweaking FDA-approved nanoparticles to work with their system. (For more on Sam's career and contributions to imaging, check out the [2018 Research Highlights](#) report.)



◀ Sanjiv Sam Gambhir  
Ludwig Stanford



Peter Sorger ▶  
Ludwig Harvard



Jia-Ren Lin ▶  
Ludwig Harvard

## A MULTIDIMENSIONAL VIEW

Cancer development and progression is a multi-step process during which the expanding cancer cell population disrupts the normal architecture of tissues. This process can influence a tumor's responsiveness and resistance to therapies. In a July [paper](#) in *eLife*, a team of researchers led by Ludwig Harvard's Peter Sorger and Jia-Ren Lin describe a simple method for generating highly multiplexed optical images of both healthy and diseased tissues. The method called t-CyCIF (short for tissue-based cyclic immunofluorescence) produces its multiplexed images based on the detection of as many as 60 antigens with antibodies as well as several stains. It employs an iterative process in which fluorescence images are repeatedly collected from the same tissue sample and then assembled into a high dimensional representation. t-CyCIF uses widely available reagents and conventional microscopes and works on formalin-fixed, paraffin-embedded glass slides—a standard preparation of tumor samples—so it can be implemented inexpensively in most research and clinical laboratories. More than 200 commercial antibodies have been tested so far for their compatibility with t-CyCIF. More information can be found at <https://www.cycif.org/> and in our interview with Peter on Page 15.

## MARKED MUTANT

Glioblastoma multiforme (GBM), the most common and deadliest type of adult brain cancer, is often driven by mutations to the epidermal growth factor receptor (EGFR). One such mutation, in alanine 289 (A289V), located within the extracellular domain of the protein, causes a particularly aggressive tumor, resulting in an average survival of just six months, as opposed to the 14 to 17 months for other versions of the cancer. A [study](#) published in July in *Cancer Cell* and led by Ludwig San Diego's Frank Furnari along with Zev Binder and Donald O'Rourke of the University of Pennsylvania explored the cause of this elevated malignancy. The researchers report that the mutation boosts the

expression of MMP1, an enzyme that increases the invasiveness of GBM. Building off a collaboration with Laura Orellana, a biophysicist and structural biologist at Stockholm University in Sweden, Frank and his colleagues explored whether a Ludwig-developed monoclonal antibody, mAb806, might shrink A289V tumors. They found it could, and that it significantly extended survival in a mouse model. Frank and his team will investigate if A289V also drives other cancers and if the antibody mAb806 might be a viable therapeutic option for those tumors as well. (For more on Frank's career and contributions to brain cancer, check out the [2018 Research Highlights](#) report.)



Frank Furnari  
Ludwig San Diego

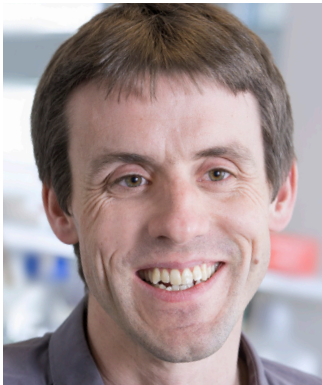


Joan Brugge  
Ludwig Harvard

## RESTORING ROS

Many tumors express unusually high levels of TRPA1—a protein nicknamed the “wasabi” receptor because its activation triggers the response that brings tears to the eyes of horseradish aficionados. A team of researchers led by Ludwig Harvard Co-director Joan Brugge recently uncovered why cancer cells are so fond of the receptor (their take on wasabi, nobody knows). They reported in a June [paper](#) in *Cancer Cell* that tumor cells use the tear-inducing TRPA1, a calcium channel protein, as a defense mechanism against a potentially lethal onslaught of reactive oxygen species (ROS)—highly

unstable and potentially lethal molecules generated in large quantities by the cancer cell's feverish metabolism. TRPA1 allows cancer cells to withstand elevated ROS by triggering a signaling cascade that inhibits apoptosis, or programmed cell death. In mice with transplanted human breast tumors, combining TRPA1 blockers and chemotherapy resulted in a significant inhibition of tumor growth. Such blockers are already under development as drugs for pain management and asthma. They might prove useful as cancer therapies as well.



David Gfeller  
Ludwig Lausanne



Michal Bassani-Sternberg  
Ludwig Lausanne



George Coukos  
Ludwig Lausanne



Panagis Filippakopoulos  
Ludwig Oxford

## DEVIATIONS FROM THE STANDARD

HLA-I molecules play a central role in antigen presentation, the process by which infected or cancerous cells inform immune cells—like killer T cells—that they should be destroyed. The HLA molecules, which come in many flavors encoded by distinct alleles, do this by offering up short fragments (peptides) of aberrant or foreign proteins (antigens) that are typically 9 to 12 amino acids in length, with canonical anchoring residues (or amino acids) at their second and last positions. A team led by Ludwig Lausanne’s David Gfeller—and including the Ludwig laboratories of Michal Bassani-Sternberg and George Coukos at the

Branch and Panagis Filippakopoulos’ lab at Ludwig Oxford—collected in-depth HLA peptidomics datasets covering 54 HLA-I alleles and developed algorithms to search for peptides that deviate from this canonical system for HLA binding. Their [results](#), reported in the *Proceedings of the National Academy of Sciences* in April, reveal that a surprisingly large fraction of HLA-I molecules can bind peptides that run on past the last, anchoring amino acid. This significantly expands the universe of possible antigens for the design of immunotherapies. The results are being put to translational use in collaboration with the Lausanne University Hospital.

## LYSIS ANALYSIS

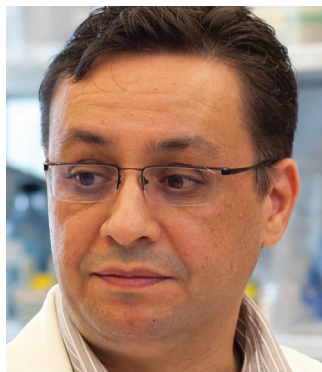
Many researchers are developing viruses that preferentially target cancer cells because infection pops cells open as virions multiply. As a bonus, this exposes the immune system to cancer antigens and can awaken potentially curative anti-tumor immune responses. This is why researchers have in parallel been exploring whether combining virotherapies with immunotherapies might optimize the treatment strategy. But do cancer cells have to be susceptible to lysis (popping open) to engage an effective immune response? Using Newcastle Disease Virus (NDV) as a model, researchers led by Ludwig MSK's Dmitry Zamarin and including Ludwig MSK's Jedd Wolchok and Taha Merghoub examined the question in a viral lysis-resistant bladder cancer model. They reported in a June [issue of \*Oncotarget\*](#) that immune-stimulating effects of NDV in bladder cancer cells are independent of a virus's ability to lyse cancer cells. When NDV was injected into a tumor and followed up with systemic checkpoint (anti-PD-1 or anti-CTLA-4) blockade, the combination engaged immune responses even in the viral lysis-resistant bladder cancer. The therapy boosted infiltration of tumors by activated immune cells and worked like a vaccine, destroying not only injected tumors but distant and untreated ones as well. This effect also improved survival of the mice.



Dmitriy Zamarin  
Ludwig MSK



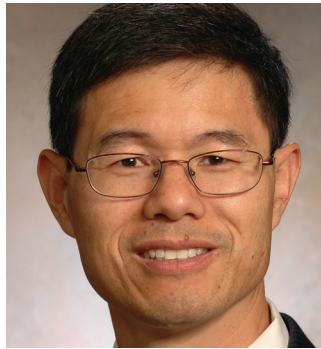
Jedd Wolchok  
Ludwig MSK



Taha Merghoub  
Ludwig MSK

## STIMULATING RESULTS

Checkpoint blockade immunotherapy doesn't work on all cancers, often because many tumor types are "cold"—insufficiently infiltrated with the T cells stimulated by such therapies. Radiotherapy, meanwhile, does engage the immune response within the targeted tumor, but doesn't induce a generalized response against all others. Combining the two has thus been a focus of much investigation. Ludwig Chicago's Wenbin Lin, Co-director Ralph Weichselbaum and their colleagues reported in a June [issue](#) of *Nature Communications* a nanotechnology-based approach to significantly enhance the synergistic effects of the combination. The researchers injected tumors in a mouse model of colon cancer with nanoparticles developed in Wenbin's lab known as Hf-based nanoscale metal-organic frameworks (nMOFs). The particles act like amplifiers of radiation, enhancing the effects of radiotherapy. Hitting the nMOF-treated tumor with low dose radiotherapy and following up with systemic anti-PD-1 checkpoint blockade elicited vigorous anti-tumor immune responses that resulted in the eradication of injected tumors and the rejection or significant regression of untreated ones. Colon cancer, notably, is not typically responsive to immunotherapy. A related radio-enhancing nanoparticle, RiMO-301, is currently in a phase 1 clinical trial to assess its safety and tolerability in patients with advanced tumors (NCT03444714).



◀ Wenbin Lin  
Ludwig Chicago



◀ Ralph Weichselbaum  
Ludwig Chicago



Matthew Vander Heiden ▶  
Ludwig MIT

## THE SKINNY ON FAT LOSS

Pancreatic ductal adenocarcinoma (PDAC) causes weight loss relatively early in its course and it has long been assumed that the degree of that loss is directly related to patient survival. In a June [paper](#) in *Nature*, a team co-led by Ludwig MIT's Matthew Vander Heiden presented results that challenge that assumption and possibly shed light on a cause of the dramatic weight loss associated with the aggressive cancer. The researchers found that even small tumors in the pancreas interfere with the organ's ability to secrete vital digestive enzymes, impairing absorption of food and prompting the mice to break down fat and other tissues, like muscle, to survive. But the weight loss doesn't seem to be indicative of prognosis. When mice were given pancreatic enzymes—as is sometimes done for human patients—they lived no longer than their unsupplemented peers. An analysis of 782 human PDAC patients similarly showed no association between degree of tissue wasting and length of survival. For now, however, their findings hold true only in mice and further study, of human patients, will be required to establish their relevance in the clinic.

## GLOWING RESULTS

When patients respond well to immunotherapy, their tumors are often teeming with T cells, especially activated ones primed for attack. But there's no easy way to determine whether that's happening and doctors have to wait weeks or months to find out whether their treatments are working. Knowing early would be helpful, even life-saving, because it would allow physicians to quickly modify treatment strategies as required. In a June [paper](#) in the *Journal of Clinical Investigation*, Ludwig Stanford's Sanjiv Sam Gambhir and his team reported an elegant technology that just might accomplish that: a radiolabeled tracer for positron emission tomography (PET) scanning that binds a protein named OX-40, which is expressed on activated T cells. They showed that it specifically reveals the location of any activated T cells in mice and demonstrated in a dual-tumor mouse model that it could, within two days, be used to predict tumor responses nine days after the initiation of an immunotherapy. It worked in the mice about 90% of the time. The technology could also be of great relevance to other diseases, including autoimmune disorders involving T cells. A clinical trial is now being planned to test it in humans. (For the story behind this research—and on Sam's career and contributions to imaging—check out the [2018 Research Highlights](#) report.)



◀ Sanjiv Sam Gambhir  
Ludwig Stanford



Samuel Hellman ▶  
Ludwig Institute  
Board Member Emeritus



Ralph Weichselbaum ▶  
Ludwig Chicago

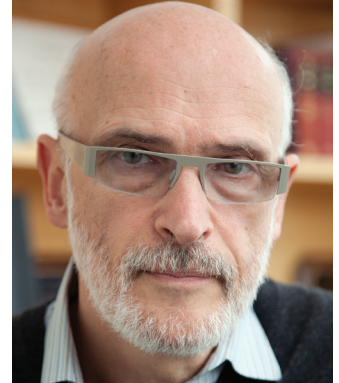
## A MIDDLE STATE

Ludwig Institute Board Member Emeritus Samuel Hellman and Ludwig Chicago Co-director Ralph Weichselbaum proposed more than two decades ago that cancers can occupy a middle state between localized growth and systemic disease. They called this state oligometastasis and have done much to define it and show that it can be cured with targeted radiotherapy or chemotherapy. In a May *Nature Communications* [paper](#), they and their colleagues reported further confirmation of their hypothesis in colorectal cancer and identified molecular patterns that can help predict patient survival. The team analyzed gene expression, microRNAs and various genomic traits in patients with oligometastatic colon and rectal cancer who had been treated with chemotherapy with surgical removal of tumors that had spread to the liver. Their analysis sorted the patients into three groups, one of which had the highest 10-year survival. Tumors from that group (Group 2), it turned out, triggered immune responses hypothesized to suppress tumor growth. The researchers reclassified the tumors using molecular and clinical data and developed an algorithm to predict survival, showing that Group 2 patients had a 94% chance of living ten years or more, compared to 45% and 10% for the other two groups. If confirmed, their findings will have significant clinical value.

## MUTUAL CARE

Regulatory T (Treg) cells suppress immune responses and help prevent autoimmunity. Though most form in the thymus, a subset of Tregs called peripheral Tregs (pTregs) differentiate elsewhere in the body and have distinct functions. This is especially true in the gut, where the commensal microbes that nourish and protect us from infection by less friendly bugs also promote the formation and accumulation of pTregs. Ludwig MSK's Alexander Rudensky and his colleagues examined how pTregs in turn influence the microbial community by comparing mice that lack pTregs with their normal relatives. They reported in a June [issue](#) of *Immunity* that a lack of intestinal pTregs perturbs the composition of gut microbiota in

mice, causing reduced body weight, dramatic changes in metabolite profiles, alterations in the soft, protective epithelial lining of the intestine and heightened anti-microbial immune responses that disrupted the early establishment of niches for bacterial colonies. Absence of a single species of bacteria, *Mucispirillum schaedleri*, can account for many of the effects. The findings suggest that pTreg cells modulate the metabolic function of intestinal microbiota by restraining immune responses that would otherwise disrupt the niches occupied by friendly microbes. (For more on Alexander's fascinating life, career and contributions to immunology, see our profile in the [2018 Research Highlights](#) report.)



Alexander Rudensky  
Ludwig MSK

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## Clinical trials

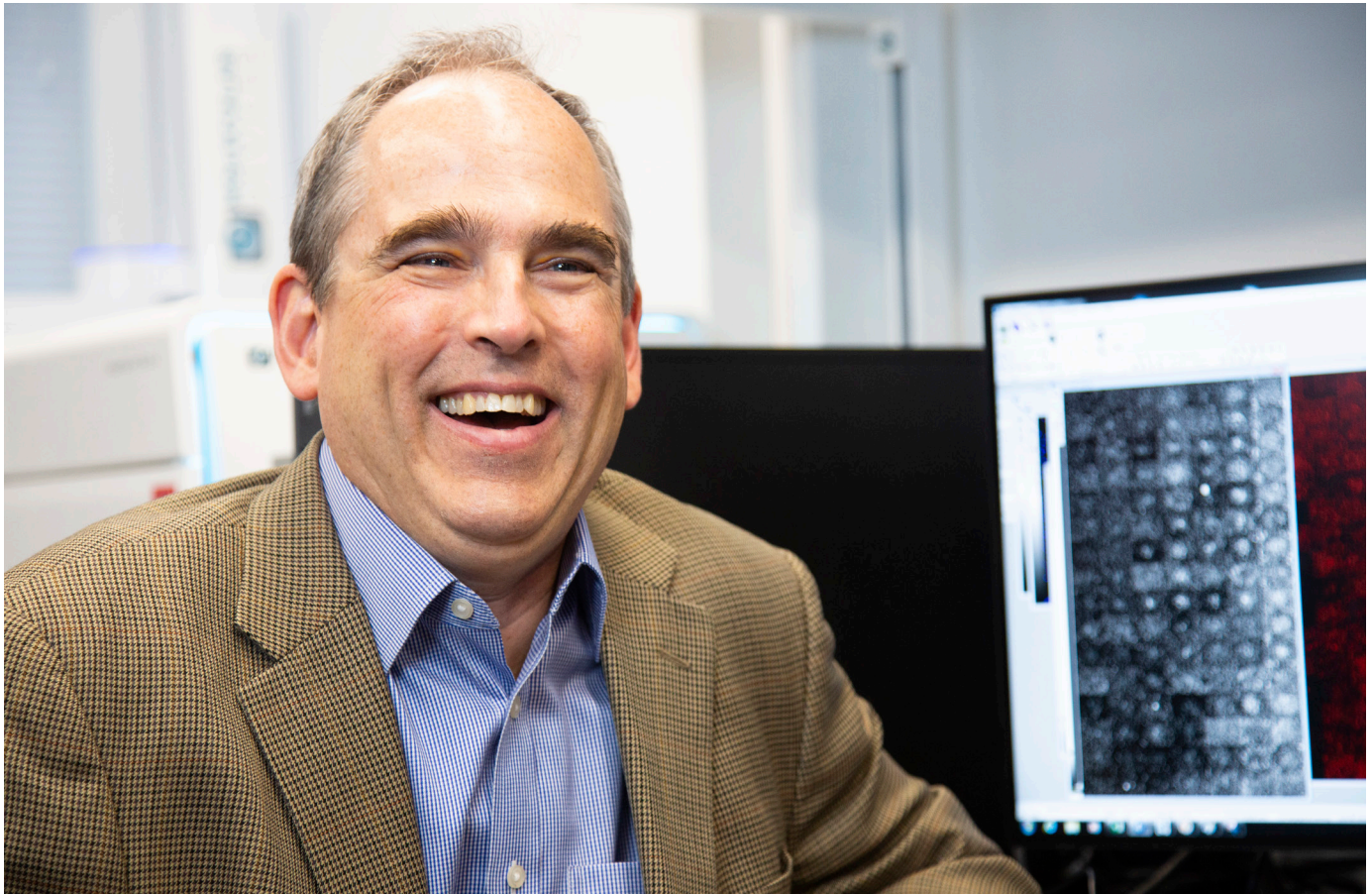


Dmitriy Zamarin  
Ludwig MSK

## GOING VIRAL, PLUS

Ludwig Cancer Research and the Cancer Research Institute (CRI) have initiated a phase 1/2 clinical trial testing a combination of the investigational viral therapy ONCOS-102, an engineered adenovirus that selectively infects and destroys cancer cells, with the checkpoint blockade antibody Imfinzi (durvalumab) in patients with advanced ovarian and colorectal cancers. Preclinical research done by Ludwig MSK's Dmitriy Zamarin

and others has shown that infecting a tumor with an "oncolytic" virus and following up with a checkpoint blockade therapy elicits an immune response that targets not only the infected tumor but also other tumors in the body. The open-label trial has completed the safety evaluation of the first patient cohort and enrollment of additional patient cohorts is ongoing.



## PETER SORGER

LUDWIG HARVARD

### **What is the focus of your current research?**

My lab focuses on the properties of single cells that predict responsiveness to natural ligands and therapeutic drugs and how understanding of these properties might improve the diagnosis and treatment of cancer. We are interested in both the average behaviors of cell populations and deviations from the average. For both normal and tumor cell populations, we find that even genetically identical cells behave quite differently at a single-cell level when exposed to drugs and ligands. In the case of resistance to many drugs, a subset of tumor cells adapts rapidly, allowing

them to survive or proliferate even when their sisters die. This adaptive response is postulated to be the origin of residual disease in cancer. Drug adaptation arises in part from normal homeostatic regulation of cell signaling and proliferation. Once we understand these adaptive and homeostatic responses, we will be able target them and improve the breadth and depth of patient responses to targeted therapies.

### **What is the tCyclF method?**

tCyclF, tissue-based cyclic immunofluorescence, is a method for constructing sub-cellular

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Breakthroughs often come from orthogonal thinking and unexpected directions. You'll have someone who isn't deeply familiar with a field taking a fresh look at a problem and finding something curious ...

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resolution, highly multiplexed images of tumors and tissue. Each “channel” in such an image represents a different molecular marker, and we can routinely perform 40- to 60-channel imaging. This is accomplished by doing the same simple thing over and over (four-color imaging) and building the complete image like a big layer cake—it's simple when each layer comes out of the oven but becomes really valuable once you assemble it and put on the frosting. We perform tCyCIF using existing instruments and reagents, making it easy for others to implement the approach.

**You're a big proponent of curiosity-driven research. How do you integrate it into your lab?**

Breakthroughs often come from orthogonal thinking and unexpected directions. You'll have someone who isn't deeply familiar with a field taking a fresh look at a problem and finding something curious about the outcome of an experiment or a clinical trial. If the results of a trial for a particular drug combination treating melanoma, for example, has a success rate of 60%, a curious observer

inevitably asks: what about the other 40%? Last December a postdoc in my group, Adam Palmer, and I published a paper in *Cell* reanalyzing data from a large number of clinical trials of combination therapies. Adam's background is antibiotic resistance not cancer therapy, and he was curious about the widespread observation that combinations of anti-cancer drugs (like antibiotic combinations) typically work better than single drugs. He showed that overall benefit likely arose from sub-sets of patients who responded to different single drugs in the combination rather than the two drugs at the same time. This model of “independent action” is very simple but has been largely ignored since it was developed 70 years ago.

Based on Adam's findings, it might often be appropriate to start with a drug mixture, since it is hard to predict response in a particular patient, and then assay an on-treatment biomarker so that only the effective drug is continued. This would reduce side effects and improve outcomes. It was curiosity-driven research by an outsider, not a systematic program of study by an expert, that provided this fresh perspective.

**Can you tell us about the Ludwig Cancer Atlas project?**

No activity is more important in the routine diagnosis of cancer than the acquisition of biopsies and their examination by pathologists. But the methods currently in use are remarkably old fashioned—it is only in the last year that the FDA has provided guidance on using computer screens rather than microscopes themselves to examine

histopathological specimens. The Tumor Atlas project will develop and deploy new approaches to histopathology that promise to revolutionize our understanding of basic cancer biology by providing highly detailed information on the molecular states of tumor, stromal and immune cells. These research applications will drive innovations in diagnostic pathology, which we expect to fully encompass and greatly extend current genomic approaches.

The first phase of the project is to create high-dimensional images of whole tumors so we can precisely locate tumor cells, supporting stroma and immune cells and determine where they interact functionally. Tumor cells will be assayed at a molecular level so we can determine the activity of cancer-causing pathways and look at possible resistance mechanisms. The second phase is taking the resulting picture data and combining the expertise of human pathologists with artificial intelligence algorithms to work out which patients will respond to a therapy or whether their cancer will progress. Right now, we are looking at dozens of tumors of six types, but we envision that in the near future the Cancer Atlas will consist of many more samples so that deep statistical analysis is possible. We will place the Ludwig Cancer Atlas in the public domain so a broad community of scientists and physicians can participate in its interpretation.

### **How does tCyclIF fit into the Cancer Atlas project?**

For the first two to three years we expect t-CyCIF to be the dominant method of



data collection for the Cancer Atlas. During that time, we will be evaluating possible alternative technologies that might be even more informative. We greatly welcome input from the Ludwig community with respect to alternative or complementary technologies. We also intend to add single cell genomic data to the Atlas, and this will also require new computational and experimental approaches.

**Peter Sorger with Ludwig  
Oxford Director Xin Lu**

### **What Ludwig sites will be involved in the project?**

We expect to engage the broad Ludwig community in the analysis of Atlas data and in making suggestions for future experiments. All of the tissues that we've processed so far have come from US hospitals, but we aim to expand this to international sites. For example, once approvals are in place, we aim to study ovarian cancer samples from George Coukos' lab in Lausanne and Barrett's esophagus samples from Xin Lu in Oxford. The Ludwig Chicago center will

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No activity is more important in the routine diagnosis of cancer than the acquisition of biopsies and their examination by pathologists. But the methods currently in use are remarkably old fashioned.

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be involved in our training programs. Implementing Atlas technology is well within the expertise of all Ludwig Centers and I suspect that some will want to perform the work locally and others will want to work with the Harvard Center.

**How do you envision the collaboration working?**

The first step is to get the workflow and analytical software working in Lausanne. The second is to process samples from Lausanne at Harvard while assessing the feasibility of performing tCyCIF in Lausanne. The third is developing panels of antibodies and analytical tools needed to get scientific insights from complex images. Ideally, we'd like to have a joint clinical fellow or an advanced postdoc who would travel back and forth between Lausanne and Harvard on a semi-regular basis. We will be evaluating this working arrangement at six- and 12-month intervals to determine if that is how we'll also proceed with Xin Lu's lab. We all intend to meet up in Lausanne this winter to evaluate progress.

**What disciplines will be involved in the effort?**

There are basically four work streams that we'll be coordinating. One involves pathologists who guide antibody selection and interpret image data—at the Ludwig Harvard Center we have eight practicing pathologists involved. Second, and equally important, are cell biologists like Joan Brugge with deep knowledge and understanding of the underlying molecular pathways. The third set of activities involves the computer science and artificial intelligence software needed

to construct and visualize a “Google Map” of human tumors and tissues. Finally, a team of analytical chemists and automation experts will ensure that we generate consistent and reliable data.

**How will it benefit the research community and ultimately the cancer patient?**

I think there are two research communities that are going to be impacted. The first is where Ludwig is really strong—translational cancer biology. And the second is the clinical trialists because we'll be able to take a deeper molecular look at the specimens currently collected as part of many clinical trials. For patients, it's the promise of getting the right drug to the right individuals. Our best guess is that even at the most advanced medical centers in the world only a third of patients really benefit from the therapy they receive. In some cases, better drugs are not yet available but in many others the challenge is matching patients to the optimal therapy. It is here that advanced histopathology could be very helpful.

Looking out eight or 10 years, we expect that multiple analytical technologies will come together in the design of better clinical trials. Our goal is to improve clinical trial design and interpretation as a way of accelerating drug development and bringing new drugs to market. This will provide new options for patients but it also promises to bring down the cost of developing new medicines, something we are exploring with the FDA and the pharmaceutical industry.

### How can interested Ludwig community members become a part of this initiative?

Ludwig members can collaborate with us using Harvard instruments or be trained here so that they can implement tCyCIF in their own labs. They can also learn it on their own from published papers and protocols. All they'll need is a microscope, antibodies and a few simple chemicals. A key component of this initiative is to make the data and the code freely available to the Ludwig community to demystify and democratize high dimensional histology.

### What would you like to accomplish in the next 5 to 10 years?

Cancer, like human physiology in general, is a complex process involving many interacting molecular processes. In general, we do not yet know how to derive actionable information from such complexity when diagnosing and treating cancer. We therefore fall back on simple rules of thumb, such as treating patients continuously at the maximum tolerated dose. My goal is to build more predictive and data driven computational models that manage this complexity and both explain and predict how cells and patients respond to therapy. These are human-in-the loop systems, in which we empower scientists and physicians to exploit a much broader range of data on biological function.

### What do you like most about being a scientist?

Ten years ago, I would have told you that it was the freedom to pursue my own ideas and the chance for reinvention. Now, it's the interaction with students and fellows that is the single most enjoyable aspect of being a scientist. Many of them have very



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interesting ideas and it's my role as mentor to help them refine these ideas and test them. I also greatly enjoy helping students and postdocs take ideas from our lab into the commercial world through start-up enterprises.

### If you could be a superhero, what would you want your superpowers to be?

Flight, so I could avoid the interminable check-in lines. Time control, to avoid missing deadlines. Telepathy, to understand what reviewer number 3 is complaining about.

### If you could have dinner with anyone from history, who would it be and why?

Alan Turing. His ideas about computation and artificial intelligence developed in the 1930s and 1940s are now finding practical implementation. It would be fascinating to get his views on the performance of a Turing Test using contemporary and future technology.

## In an era of personalized medicine, are big randomized clinical trials an endangered species?



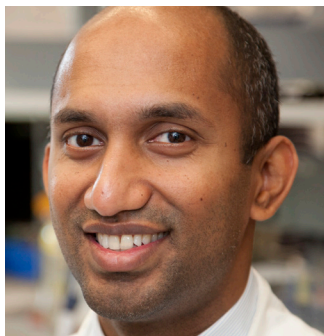
A multitude of different tumor/immune constellations can be therapeutically exploited. This has resulted in the development of new therapies designed for use in smaller patient populations selected with the use of biomarkers to better personalize treatment. However, smaller trials will not have the safety profile we expect from large trials, which may increase therapeutic risks as well as the liability of physicians and drug makers.

**PAUL SCHWARZENBERGER**  
Ludwig Institute



There is a critical need to integrate molecular and genetic information to inform personalized approaches for cancer patients. As precision oncology continues to evolve, I believe large clinical trials will continue to identify practice-changing treatments and serve as platforms from which to discover the biological determinants that predict treatment efficacy.

**SEAN PITRODA**  
Ludwig Chicago



Large randomized trials have an important role in medicine and will continue to be necessary, particularly for studies that impact large numbers of individuals. For molecularly defined diseases, smaller trials based on molecular characteristics are likely to be more pertinent than large trials in which an effect could be obscured.

**CHETAN BETTEGOWDA**

Ludwig Johns Hopkins



Randomized clinical trials have been a cornerstone of modern medical progress and, I believe, will continue to play an important role. However, personalized medicine approaches can elicit dramatic and previously unprecedented treatment effects that, when achieved, undoubtedly outperform existing standards. In these situations, a strict adherence to randomized studies may not only be impractical, due to the rarity of the population, but also unethical.

**DAVID HYMAN**

Early Drug Development Service, MSK

## Required reading

### Ludwig Chicago

**Nature Communications**  
**2018 June 15**  
**Nanoscale metal-organic frameworks enhance radiotherapy to potentiate checkpoint blockade immunotherapy.**

Ni K, Lan G, Chan C, Quigley B, Lu K, Aung T, Guo N, La Riviere P, Weichselbaum RR, Lin W.

**Nature Communications**  
**2018 May 4**

**Integrated molecular subtyping defines a curable oligometastatic state in colorectal liver metastasis.**

Pitroda SP, Khodarev NN, Huang L, Uppal A, Wightman SC, Ganai S, Joseph N, Pitt J, Brown M, Forde M, Mangold K, Xue L, Weber C, Segal JP, Kadri S, Stack ME, Khan S, Paty P, Kaul K, Andrade J, White KP, Talamonti M, Posner MC, Hellman S, Weichselbaum RR.

### Ludwig Harvard

**Proceedings of the National Academy of Sci USA 2018**  
**August 27 [Epub ahead of print]**

**Mechanisms of enhanced drug delivery in brain metastases with focused ultrasound-induced blood-tumor barrier disruption.**

Arvanitis CD, Askoxylakis V, Guo Y, Datta M, Kloepper J, Ferraro GB, Bernabeu MO, Fukumura D, McDannold N, Jain RK.

**Elife 2018 July 11**

**Highly multiplexed immunofluorescence imaging of human tissues and tumors using t-CyCIF and conventional optical microscopes.**

Lin JR, Izar B, Wang S, Yapp C, Mei S, Shah PM, Santagata S, Sorger PK.

**Cancer Cell 2018 June 11**

**Cancer cells co-opt the neuronal redox-sensing channel TRPA1 to promote oxidative-stress tolerance.**

Takahashi N, Chen HY, Harris IS, Stover DG, Selfors LM, Bronson RT, Deraedt T, Cichowski K, Welm AL, Mori Y, Mills GB, Brugge JS.

### Ludwig Lausanne

**Proceedings of the National Academy of Sciences USA 2018**  
**May 15**

**The C-terminal extension landscape of naturally presented HLA-I ligands.**

Guillaume P, Picaud S, Baumgaertner P, Montandon N, Schmidt J, Speiser DE, Coukos G1, Bassani-Sternberg M, Filippakopoulos P, Gfeller D.

### Ludwig MIT

**Molecular Cancer Therapeutics**  
**2018 August 10 [Epub ahead of print]**

**iRGD-guided tumor-penetrating nanocomplexes for therapeutic siRNA delivery to pancreatic cancer.**

Lo JH, Hao L, Muzumdar MD, Raghavan S, Kwon EJ, Pulver EM, Hsu F, Aguirre AJ, Wolpin BM, Fuchs CS, Hahn WC, Jacks T, Bhatia SN.

**Nature 2018 June**

**Altered exocrine function can drive adipose wasting in early pancreatic cancer.**

Danai LV, Babic A, Rosenthal MH, Dennstedt EA, Muir A, Lien EC, Mayers JR, Tai K, Lau AN, Jones-Sali P, Prado CM, Petersen GM, Takahashi N, Sugimoto M, Yeh JJ, Lopez N, Bardeesy N, Fernandez-Del Castillo C, Liss AS, Koong AC, Bui J, Yuan C, Welch MW, Brais LK, Kulke MH, Dennis C, Clish CB, Wolpin BM, Vander Heiden MG.

### Ludwig MSK

**Cell 2018 August 23**  
**Single-Cell Map of Diverse Immune Phenotypes in the Breast Tumor Microenvironment.**

Azizi E, Carr AJ, Plitas G, Cornish AE, Konopacki C, Prabhakaran S, Nainys J, Wu K, Kisieliovas V, Setty M, Choi K, Fromme RM9, Dao P, McKenney PT, Wasti RC, Kadaveru K, Mazutis L, Rudensky AY, Pe'er D.

**Oncotarget 2018 June 19**

**Lysis-independent potentiation of immune checkpoint blockade by oncolytic virus.**

Oseledchik A, Ricca JM, Gigoux M, Ko B, Redelman-Sidi G, Walther T, Liu C, Iyer G, Merghoub T, Wolchok JD, Zamarin D.

**Immunity 2018 June 19**

**Extrathymically generated regulatory T cells establish a niche for intestinal border-dwelling bacteria and affect physiologic metabolite balance.**

Campbell C, Dikiy S, Bhattarai SK, Chinen T, Matheis F, Calafiore M, Hoyos B, Hanash A, Mucida D, Buccia V, Rudensky AY.

### Ludwig San Diego

**Nature Structural & Molecular Biology 2018 August**  
**Identification of Exo1-Msh2 interaction motifs in DNA mismatch repair and new Msh2-binding partners.**

Goellner EM, Putnam CD, Graham WJ 5th, Rahal CM, Li BZ, Kolodner RD.

**Cancer Cell 2018 July 9**

**Epidermal growth factor receptor extracellular domain mutations in glioblastoma present opportunities for clinical imaging and therapeutic development.**

Binder ZA, Thorne AH, Bakas S, Wileyto EP, Bilello M, Akbari H, Rathore S, Ha SM, Zhang L, Ferguson CJ, Dahiya S, Bi WL, Reardon DA, Idubai A, Felsberg J, Hentschel B, Weller M, Bagley SJ, Morrisette JJD, Nasrallah MP, Ma J, Zanca C, Scott AM, Orellana L, Davatzikos C, Furnari FB, O'Rourke DM.

### Ludwig Stanford

**Journal of Clinical Oncology**  
**2018 August 20 [Epub ahead of print]**

**Circulating tumor DNA measurements as early outcome predictors in diffuse large B-cell lymphoma.**

Kurtz DM, Scherer F, Jin MC, Soo J, Craig AFM, Esfahani MS, Chabon JJ, Stehr H, Liu CL, Tibshirani R, Maeda LS, Gupta NK, Khodadoust MS, Advani RH, Levy R, Newman AM, Dührsen U, Hüttmann A, Meignan M, Casasnovas RO, Westin JR, Roschewski M, Wilson WH, Gaidano G, Rossi D, Diehn M, Alizadeh AA.

**Nature Biomedical Engineering**  
**2018 July 16**

**An intravascular magnetic wire for the high-throughput retrieval of circulating tumour cells in vivo.**

Vermesh O, Aalipour A, Ge TJ, Saenz Y, Guo Y, Alam IS, Park SM, Adelson CN, Mitsutake Y, Vilches-Moure J, Godoy E, Bachmann MH, Ooi CC, Lyons JK, Mueller K, Arami H, Green A, Solomon EI, Wang SX, Gambhir SS.

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## Required reading

### **Journal of Clinical Investigation** **2018 June 1**

**Imaging activated T cells predicts response to cancer vaccines.**

Alam IS, Mayer AT, Sagiv-Barfi I, Wang K, Vermesh O, Czerwinski DK, Johnson EM, James ML, Levy R, Gambhir SS.

### **Ludwig Wistar**

#### **Cell 2018 June 28**

**Acid suspends the circadian clock in hypoxia through inhibition of mTOR.**

Walton ZE, Patel CH, Brooks RC, Yu Y, Ibrahim-Hashim A, Riddle M, Porcu A, Jiang T, Ecker BL, Tameire F, Koumenis C, Weeraratna AT, Welsh DK, Gillies R, Alwine JC, Zhang L, Powell JD, Dang CV.

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