LUDWIG LINK

APRIL 2020

IN THIS ISSUE

LUDWIG CANCER RESEARCH

LIFE-CHANGING SCIENCE

LUDWIG LINK

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LETTER

This issue of Ludwig Link arrives at a personally and professionally challenging time for everyone in the Ludwig community and beyond. Aside from coping with the worry caused by the COVID-19 pandemic and its economic fallout, many of you have had to secure your labs and, at the same time, work to assure that critical research resources are preserved and protected. We appreciate that this is a time of tremendous uncertainty for everyone and wish you all the best in your efforts. We recognize that you are all doing your part to help end this pandemic and following whatever mitigating and safety measures your host institution requires.

If the New York office can be of help to you in your efforts, please do not hesitate to contact us.

We hope you and your loved ones are staying safe and healthy in this difficult time. Your work will resume when the pandemic passes, and we hope this newsletter will remind you of how important it is to the wellbeing of humanity. You will, we're sure, find ample evidence of that in the following pages, which brim with intriguing discoveries, awards and honors bestowed on your colleagues and include an engaging interview with a cancer research pioneer.

Sincerely,

Rachel Reinhardt
Senior Vice President for Communications

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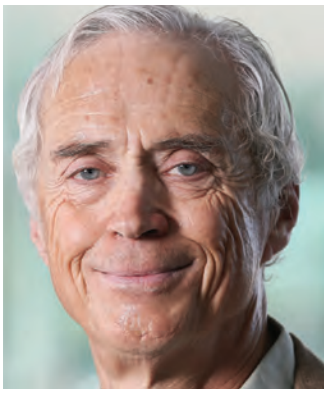


Douglas Hanahan
Ludwig Lausanne

WELCOMING A LUDWIG DISTINGUISHED SCHOLAR

Douglas Hanahan was appointed Ludwig Distinguished Scholar at the Lausanne Branch of the Ludwig Institute for Cancer Research in January. Doug has made many landmark contributions to cancer biology and immunology. Working out of the Cold Spring Harbor Laboratory in the late 80s, he developed one of the first transgenic mouse models of cancer and used it to investigate the stages of cancer progression, the role of oncogenes in that process and the immune system's response. Collaborating with the late Judah Folkman, he also used these mouse models to identify the "angiogenic switch" employed by tumors to generate new blood vessels. Their subsequent work in this area led to the development of new anti-angiogenic drugs. Doug's seminal review *The Hallmarks of Cancer*, authored with Ludwig MIT Co-director Robert Weinberg and published in *Cell* in 2000, drew from all corners of basic cancer research to create a unifying conceptual framework for understanding tumor initiation and progression. It is widely regarded among the most influential publications of modern cancer biology. His laboratory continues to explore the stages and drivers of tumor progression and pharmacologic strategies to disrupt them.

Don Cleveland
Ludwig San Diego

FOR TAKING ON ALS

Ludwig San Diego's Don Cleveland and colleagues received the inaugural Healey Center International Prize for innovation in amyotrophic lateral sclerosis (ALS) research for their discovery and development of antisense oligonucleotide (ASO) technology as a treatment approach for SOD1-mediated ALS. Over 100 different mutations to the SOD1 gene have been linked to inherited ALS, and ASO intervention is already being evaluated in clinical trials. The approach holds promise for other forms of ALS and a number of other neurodegenerative disorders as well. Don has made many significant discoveries on the molecular biology of neurodegenerative diseases, including the identification of tau, a protein that features in other cognitive disorders, including Alzheimer's disease, frontal temporal dementia and chronic traumatic brain injury. Don and his colleagues received their award during the 30th International Symposium on ALS/MND in Perth, Australia on December 6th. Click here to see Don's presentation at the award ceremony.

FOR MINIATURE MIRACLES

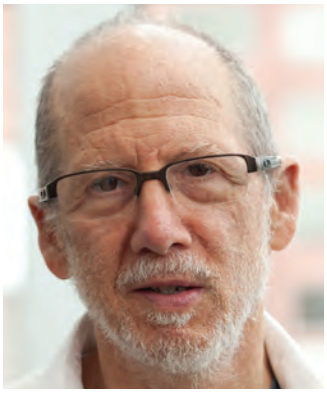
Ludwig MIT's Sangeeta Bhatia has been elected to the National Academy of Medicine. She is already a member of the National Academies of Science and of Engineering, making her just the 25th person to be elected to all three national academies. Sangeeta and her colleagues harness miniaturization tools from the world of semiconductor manufacturing to improve human health, and have designed nanoparticles and other materials to diagnose and treat disease, including cancer. Her laboratory also uses computer-chip technology to fabricate synthetic microtissues to model and treat diseases of the liver. Sangeeta and her trainees have founded several biotechnology companies to further develop these technologies. Election to the National Academy of Medicine is considered one of the highest honors in the fields of health and medicine and recognizes individuals who have demonstrated outstanding professional achievement and commitment to service.

Sangeeta Bhatia
Ludwig MIT

FOR IMMUNOMETABOLIC INSIGHT

Ludwig Lausanne's Ping-Chih Ho, newly appointed Ludwig Associate Member, was selected by the European Molecular Biology Association (EMBO) for membership in its Young Investigator Program. Ping-Chih is a leader in the field of immunometabolism, which explores how cellular nutrients and byproducts of metabolism mediate a molecular conversation between the immune system and the tissues it patrols. His lab has made significant contributions to our understanding of how immunometabolomic effects alter the behavior of immune cells like macrophages in tumors, disrupt the T cell response to cancer and how those escape mechanisms might be pharmacologically undone for immunotherapy. EMBO Young Investigators are researchers under the age of 40 who are within their first four years as group leaders and have a proven record of scientific excellence. As part of the program, Ping-Chih will receive training in leadership skills and responsible research practices, and have access to core facilities at the European Molecular Biology Laboratory in Heidelberg, Germany.

Ping-Chih Ho
Ludwig Lausanne



Bert Vogelstein
Ludwig Johns Hopkins

FOR MODEL SCIENCE

Ludwig Johns Hopkins Co-director Bert Vogelstein was awarded the 2020 Jessie Stevenson Medal for his transformative work illuminating the fundamental principles and molecular drivers of cancer initiation and progression, which laid the groundwork for more sophisticated approaches to diagnosing and treating cancers. The multi-step model of cancer progression he developed more than three decades ago, popularly known as the "Vogelgram," is now taught around the world. His subsequent work charting the full spectrum of genes expressed in scores of malignancies has left a mark on virtually every aspect of basic and translational cancer research. Today, his lab mainly focuses on developing novel diagnostic methods for the earlier detection of cancers and new gene-based therapeutic approaches for treating advanced cancers. The Jessie Stevenson Kovalenko Medal is awarded by the National Academy of Sciences every two years for outstanding research in the medical sciences.

Alexander Rudensky
Ludwig MSK

FOR ENRICHING IMMUNOLOGY

Ludwig MSK's Alexander Rudensky is the 2020 recipient of the AAI-Thermo Fisher Meritorious Career Award. He received the award, presented by the American Association of Immunologists, for his contributions to the field of immunology. Alexander is an internationally recognized leader in the study of regulatory T cells (Tregs), which control immune responses, prevent autoimmunity and participate in wound healing. His work has touched on almost every aspect of Treg cells, from the molecular biology and genetics of their generation, maintenance and function to their relevance to cancer biology—in which they play a central role in thwarting the immune surveillance of tumors. Alexander's discoveries hold significant promise for the development of therapies for autoimmune diseases and the prevention and treatment of cancer. He will present a lecture titled *The virtue of restraint in adaptive immunity* in May, when he receives his award in Honolulu, Hawaii.

DEADLY DUO UNDONE

The main risk factor for stomach cancer is an infection by Helicobacter pylori (H. pylori). Strains of H. pylori that produce the toxin CagA significantly increase the risk of stomach cancer compared with strains that do not. In a January paper in the *Proceedings of the National Academy of Sciences*, Ludwig Oxford's Xin Lu and colleagues found that interaction between the tumor suppressor ASPP2 and CagA is a key event in disruption of cell polarity—the asymmetric organization of cells—during H. pylori infection. Interfering with the CagA–ASPP2 interaction using small molecules or a specific peptide blocks loss of cell polarity and reduces bacterial colonization in organoids, which are tiny, self-organized three-dimensional tissue cultures derived from stem cells, in this case from the stomach. The results suggest that agents that can prevent changes in host-cell polarity upon bacterial infection might represent a new class of antimicrobial agents.

Xin Lu
Ludwig Oxford

A MATTER OF FAT

Obesity is something of a paradox in kidney cancer. While a very high body-mass index increases the risk of developing kidney cancer, obese people are also more likely to survive the disease than those of normal weight. A study supported by the Ludwig Center at MSK and led by Ari Hakimi of MSK and published in *Lancet Oncology* in December shed light on how obesity may actually benefit people undergoing treatment. The team studied fat samples taken for research purposes from patients undergoing surgery for renal cell carcinoma (RCC) and found that the fat tissue surrounding kidney tumors plays a critical role in how well a treatment works. They found that the fat in obese people is more inflamed than in people of normal weight, and that this draws immune cells to the site of the tumor to attack it. Yet, surprisingly, the kidney tumors themselves did not appear to be stimulating an immune response, as they were cold—that is, relatively unpopulated with anti-cancer immune cells. Ari and his colleagues are now further exploring the immune response they detected and looking for biomarkers that might flag kidney tumors as susceptible to immunotherapy.

Ari Hakimi
Ludwig MSK

Rakesh Jain
Ludwig Harvard

SUPPRESSOR SUPPRESSION

Immune checkpoint inhibitors are variously effective in treating many types of cancer. But the brain cancer glioblastoma multiforme (GBM) is not one of them, at least not so far. Ludwig Harvard's Rakesh Jain co-led a study reported in the *Proceedings of the National Academy of Sciences* in December that examined a new strategy to make GBM susceptible to immune checkpoint inhibitors. The approach targets chemokine receptors, which draw myeloid-derived suppressor cells (MDSCs)—inhibitors of anticancer immune responses—to the regions around GBM tumors. Rakesh and his team initially looked at mice bred to develop GBM and lack the chemokine receptor 2 (CCR2). They showed that the regions around the GBM tumors lacked MDSCs and that the tumors themselves were susceptible to immune responses induced by checkpoint blockade. They also demonstrated that in mice expressing CCR2, treatment with a molecule that blocks the receptor had similar effects. The results support targeting of CCR2-expressing myeloid cells to enhance immunotherapies for GBM.

Peter Sorger
Ludwig Harvard

A BROADER ATTACK

Current treatments for triple negative breast cancer (TNBC) are associated with high rates of relapse and low overall survival. Many drug development efforts for this deadly cancer have focused on inhibiting the PI3K signaling pathway, whose components are often mutated and overactive in TNBCs. But such therapies have been only moderately successful. In a December paper in *Cell Systems*, Ludwig Harvard investigators led by Peter Sorger systematically analyzed responses of TNBC cells to a diverse collection of PI3K pathway inhibitors. They found that one drug, Torin2, is unusually effective because it inhibits both mTOR, a central hub of PI3K signaling pathways, and a variety of PI3K-like kinases (PIKKs) to target vulnerabilities exposed when the proliferating cancer cell replicates its DNA. The researchers demonstrate that Torin2 represents a mechanistically distinct and uniquely cytotoxic class of PI3K pathway inhibitors. The findings have the potential to be translated therapeutically by the development of Torin2 analogs or combinations of existing mTOR and PIKK inhibitors.

A MALIGNANT BALANCE

The biochemical pathways altered in cancer cells can have a variety of effects, not all of them good for the cell in question. This suggests that as such alterations accumulate in cancer cells, only those altered pathways whose consequences are in harmony with that of others allow the cell to survive and drive tumor growth. Ludwig Oxford's Peter Ratcliffe and colleagues examined this hypothesis in renal clear cell carcinoma (RCC), a cancer driven by the constitutive activation of a key regulator of the hypoxic response, HIF. In particular, they examined the overlap between DNA-binding elements involved in the HIF pathway and variations in DNA sequences that affect cancer susceptibility. Peter and his team published in *Scientific Reports* in December their comparative analysis of that overlap in RCC and in other cancers in which HIF is differently activated—say, by oxygen starvation. Their findings support a "pathway tuning" model of cancer, in which precise modulation of multiple outputs of specific, activated biochemical pathways is critical to the emergence of cancer. This implies that selective pressures balance the effects of a vast network of interacting pathways during cancer development to generate a viable cancer cell and, ultimately, a tumor. This, the researchers argue, should focus attempts to identify the nature and consequences of those selective pressures.

Peter Ratcliffe
Ludwig Oxford

MICROHELPER

Adoptive cell therapies for cancer involve the extraction of T cells from patients and their infusion back into the donors after their selective expansion in culture. But even T cells that are highly reactive to cancer cells can be thwarted by the immunosuppressive environment within tumors. In a December paper in *Molecular Therapy Oncolytics*, graduate student Gwennaelle Monnot and a team of investigators led by Pedro Romero of Ludwig Lausanne reported their findings on the potential of using miR-155—a small regulatory noncoding RNA known to play a role in killer T cell fitness—to improve the persistence and functionality of adoptively transferred T cells. They showed that overexpression of the microRNA in tumor antigen-specific T cells bolsters their metabolic fitness and functionality within the tumor and improves, in mice, the control of B16 melanoma tumors that express an antigen weakly recognized by T cells. Overexpression of miR-155, they suggest, could generally improve adoptive T cell therapies and, further, expand the use in such therapies of weakly reactive anti-tumor T cells naturally generated by patients.

Pedro Romero
Ludwig Lausanne

CAR-T REVIVAL

CAR T-cell therapy has racked up remarkable wins, but it does have its limitations. For one thing, while it works against some blood cancers, it hasn't yet been shown to be effective against solid tumors. For another, its efficacy can wane after some time even against blood cancers, often due to the T cell exhaustion that naturally kicks in after prolonged T cell activity. In a December *Nature* paper, researchers led by Ludwig Stanford's Crystal Mackall report their exploration of the phenomenon, and an approach to its prevention. Using a technique called ATAC-Seq—developed in the laboratory of Ludwig Professor Howard Chang—the researchers explored differences in the activation of the genome between exhausted and active T cells. This revealed an imbalance in the activity of a class of genes that regulate protein levels in the exhausted cells, which led in turn to the inhibition of T-cell activity. The team engineered CAR T-cells to overexpress c-Jun, a protein involved in T-cell activation, and demonstrated in preclinical models of leukemia, and even a (solid) bone cancer, that the modified CAR-T cells could better target cancer than their ordinary counterparts, further extending the lives of mice.

Crystal Mackall
Ludwig Stanford

Howard Chang
Ludwig Stanford

Benoît Van den Eynde
Ludwig Institute

TRACKING TDO

Certain enzymes expressed by tumors disable T cells by depriving them of a key amino acid known as tryptophan. The expression of one of them, IDO, has been exhaustively studied. But little is known about the expression of the other, TDO, or about its effects on the immune system. In a December paper in *Cancer Immunology Research*, a team led by Ludwig's Benoît Van den Eynde reported their use of a novel TDO-specific monoclonal antibody to explore TDO's role in cancer biology. Benoît and colleagues showed that TDO is expressed by the majority of human cancers and plays an immunosuppressive role in hepatocellular carcinoma—the most common type of primary liver cancer—where it could be a prime immunotherapeutic target. They found that approximately 25% of glioblastomas and 10% of kidney cancers also expressed TDO in tumor cells. In many other cancers, TDO seems to be involved not so much in immune suppression as in angiogenesis, or the generation of tumor-feeding blood vessels. In either case, the enzyme does appear to hold some promise as a target for cancer therapy.

DESIGNING AND TUNING SIGNALS

Allostery is a mechanism by which proteins and other biological molecules bind to a protein site other than its active site to indirectly regulate its activity. Ludwig Lausanne's Patrick Barth and his team have developed a computational method for both predicting and designing such allosteric functions in proteins. Reported in December in *Nature Chemical Biology*, their method permits the accurate tuning and even re-purposing of protein functions via allostery. Patrick and his colleagues proved the efficacy of their approach by engineering a broad spectrum of novel signaling functions into G protein-coupled receptors (GPCRs). They made 36 variants of the dopamine receptor D2, which regulates cognition and is a target of antipsychotic drugs and achieved a greater than 80% success rate in their designs. Their model predicted the signaling effects of more than 100 known mutations to GPCRs. One variant of D2 they designed was responsive to an entirely different neurotransmitter, serotonin. The technology is likely to be of significant utility in synthetic biology and cell engineering.

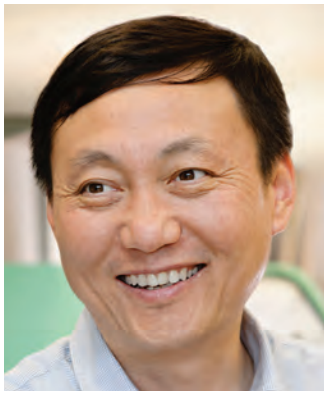
Patrick Barth
Ludwig Lausanne

TWO-HIT STRATEGY

Highly aggressive childhood brain cancers known as diffuse midline gliomas (DMGs)—including intrinsic pontine glioma (DIPG) and spinal cord glioma, among others—are diagnosed every year in a few hundred children, typically between the ages of 4 to 12. In a November paper in *Science Translational Medicine*, Ludwig Stanford researcher Michelle Monje and colleagues reported preclinical results showing that a new drug combination—panobinostat (a histone deacetylase inhibitor) and marizomib (a proteasome inhibitor)—could offer some hope for treating these incurable cancers. Previous work suggested that the drug panobinostat kills DMG cells, but the tumors tend to become resistant to the treatment. The team tested 2,706 single compounds and 9,195 drug combinations in cell cultures from patients' tumors and found that the two-drug combination increased survival in mice implanted with tumors derived from those patient samples. It also interfered with the cancer cells' ability to make a compound known as NAD, which plays a central role in cellular metabolism and energetics. Though NAD is essential to the survival of any cell, the two drugs synergize in a way that selectively affects NAD availability only in cancer cells. The researchers are now designing a clinical trial for the drug combination and for marizomib alone.

Michelle Monje
Ludwig Stanford

Paul Mischel
Ludwig San Diego



Bing Ren
Ludwig San Diego

Howard Chang
Ludwig Stanford

HYPERACTIVE NUCLEAR HOOPS

A study led by Ludwig San Diego's Paul Mischel and Bing Ren with Ludwig Stanford's Howard Chang took a deep dive into how extrachromosomal DNA (ecDNA) drives tumor growth, heterogeneity and drug resistance. Circles of DNA that exist outside chromosomes and encode multiple copies of growth-driving genes, ecDNAs are almost exclusively found in cancer cells. Previous work in Paul's lab uncovered the phenomenon and revealed that ecDNA plays a key role in tumor progression and cellular heterogeneity across a large variety of cancers. In their most recent study, the team investigated the link between the molecular structure and gene expression patterns of ecDNAs using a variety of genomic analysis tools. They reported in *Nature* in November that though ecDNA is wound tightly around protein cores, it is structured in a manner that makes its cancer-driving genes highly accessible and prone to extraordinarily high levels of expression. The pitched expression of oncogenes in turn helps tumor cells evolve more quickly and respond more nimbly to changing environments and threats like cancer therapy.

DEADLY LOW PROFILE

Although a small subset of colorectal cancers that have highly mutated DNA respond briskly to checkpoint inhibitors, most have proved highly resistant to these immunotherapies. A team of researchers led in part by Ludwig Lausanne investigator Michal Bassani-Sternberg applied a technology known as immunopeptidomics to small patient-derived tumors, or organoids, grown in the lab to explore the reason for this resistance. Michal and her team also sought to determine whether resistance could be reversed by treatment with IFN-gamma or MEK inhibitors. They reported in the *Journal for ImmunoTherapy of Cancer* in November that advanced colorectal cancers display very few neoantigens—mutated protein fragments detected by patrolling T cells—on their surface. This helps explain why immunotherapies that stimulate a T cell attack have not worked well in the majority of advanced CRCs. The researchers looked at five mini-tumors grown from patient samples, which together contained 612 gene mutations that could potentially generate a neoantigen. Only three neoantigens were detected—just a fraction of the number predicted by computational methods. Neither IFN-gamma nor MEK inhibitors improved the presentation of neoepitopes in the organoids.

Michal Bassani-Sternberg
Ludwig Lausanne

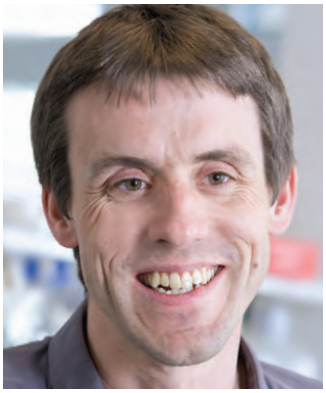
CLOCK LINK

The Myc gene encodes a protein that broadly orchestrates the gene expression essential to metabolism. It is also among the most frequently mutated, overexpressed or otherwise dysregulated genes in cancer. Studies led by Ludwig Scientific Director Chi Van Dang have previously shown that Myc dysfunction alters cellular metabolism to fuel tumor growth and also has a profound impact on the circadian rhythms of cancer cells. In a study published in *Cell Reports* in November, Chi, his collaborator Amita Segal and colleagues showed that Myc's role in linking metabolism to the biological clock holds true in fruit flies as firmly as it does in humans and their cancers. Overexpression of the *Drosophila* Myc protein (dMyc) significantly disrupted not only the metabolism of fruit flies, but their circadian behavior as well. The latter disruption was accompanied by the heightened expression of genes involved in circadian rhythms—*cyc, tim, cry* and *cwo*. Mutations that dialed down Myc activity similarly influenced drosophila circadian behaviors, but these could be reversed by the loss of a protein known as dMnt, which suppresses dMyc activity. The findings expose the deep evolutionary roots of Myc's role in linking metabolism and the circadian clock.

Chi Van Dang
Ludwig Institute

Julien Racle
Ludwig Lausanne

Michal Bassani-Sternberg
Ludwig Lausanne



David Gfeller
Ludwig Lausanne

EMPOWERING PREDICTIONS

Helper T cells play a central role in orchestrating adaptive immune responses and have been shown in recent studies to be essential to the elicitation of therapeutic anti-tumor immune responses by cancer vaccines. In an October paper in *Nature Biotechnology*, Ludwig Lausanne researchers Julien Racle, Michal Bassani-Sternberg and David Gfeller reported a new and more accurate method to identify the molecular signs of cancer likely to be presented to helper T cells. To develop their model, the researchers applied mass spectrometry and determined the amino acid sequences of more than 99,000 HLA-II binding peptides eluted from cells and tissues. With this data, their novel computational tool based on machine learning, called MoDec (for motif deconvolution), accurately defined HLA-II consensus binding motifs. The results were used to train an algorithm to predict the HLA-II presentation capability of peptides from a variety of tumors and pathogens. The predictive power of their method is at least twice as good as previous techniques and is being employed in Ludwig Lausanne's efforts to develop individualized immunotherapies for cancer.

THE TRIBES OF DC

Dendritic cells (DCs) patrol the body and alert the adaptive immune system to pathogens or cancerous cells. They have traditionally been divided into two tribes, cDC1 and cDC2, based on molecular markers and their priming of distinct T cell responses. cDC1 cells appear to be uniform, have been well characterized and are known to provoke killer T cell responses. But cDC2 cells were less well understood. A study led by Ludwig MSK Director Alexander Rudensky and published in *Cell* in October just changed that. Alexander and his team profiled gene expression in thousands of individual cDC2 cells from mice and found that this DC tribe consists of two distinct clans, now named cDC2A and B. The former seems anti-inflammatory and primarily concerned with wound-healing; the latter tends to direct inflammatory T cell responses. Alexander and his team found that human cDC2 cells shared these same divisions. The findings suggest that the two cDC2 clans could play opposing roles in the development of cancer—cDC2A cells, for example, could be nurturing tumor growth, while cDC2B could be targeting cancer cells. In support of this possibility, the researchers found cDC2A cells hiding out in melanoma tumors taken from patients. The work opens a door to the design of new immunotherapies.

◀ Alexander Rudensky
Ludwig MSK

George Demetri ▶
Ludwig Harvard

Bradley Bernstein ▶
Ludwig Harvard

ONCOGENIC ALTERNATIVES

Most gastrointestinal stromal tumors (GISTs), a type of connective tissue cancer, respond to therapies that target the mutated enzymes driving their growth. But 10% to 20% of such tumors have no driver mutations in their genome. Researchers led by Ludwig Harvard Co-director George Demetri and investigator Bradley Bernstein have identified what drives these cancers and shown, in preclinical studies, how they might be treated. Their findings reveal how epigenetic changes—chemical modifications to DNA and its protein packaging that alter how genes are read, not abnormalities in the gene sequences themselves—can lead to the development of GISTs and other cancers. An epigenetic abnormality dismantles an element of chromosomal structure (an insulator) that prevents a cancer-promoting growth factor gene, FGF4, from contacting a stretch of DNA that serves as an "on" switch for gene expression. The researchers also showed how another epigenetic abnormality similarly causes aberrant contacts between an on-switch and the KIT gene that is activated by somatic mutations in the majority of GIST patients. They demonstrated that human GISTs growing as patient-derived xenografts in mice could be suppressed with FGF receptor inhibitors, either alone or in combination with a standard kinase inhibitor therapy for GIST called sunitinib. The work was published in October in *Nature*.

THE ADVANTAGE OF SLOWING DOWN

Ludwig Johns Hopkins Co-director Bert Vogelstein and and Johns Hopkins colleague Cristian Tomasetti led a study exploring why cancer incidence climbs precipitously after the age of 65 but then declines in people over the age of 80. A major clue for the study came from a review of data that indicated the accumulation of mutations slows in the very old. Cristian, Bert and their colleagues analyzed cell replication rates in samples of healthy, self-renewing tissues collected during biopsies and other medical procedures from more than 300 patients in their 20s and 80s.

They reported in a September paper in the *Proceedings of the National Academy of Sciences* that cell division rates slowed by 40% in colon tissue samples from people in their 80s compared with those in their 20s. In esophageal tissue, the division rate slowed by about 25%, by 26% in the duodenum and by 83% in tissue found near the nose. A parallel slowdown was not seen in mice, which do not experience a similar decline in cancer incidence. These results have significant implications for understanding the relationship between normal stem cells, aging and cancer.

Bert Vogelstein
Ludwig Johns Hopkins

ELECTRIC DISCOVERY

A team led by Michelle Monje of the Ludwig Center at Stanford has discovered that brain tumors known as high-grade gliomas form synapses—or connections with neurons—and tap electrical signals from healthy neurons to drive their own growth. Reported in September in *Nature*, the study further revealed that many of the cancer cells within the brain tumors also contain cell-to-cell electrical connections known as gap junctions, which help transmit and amplify impulses. High-grade gliomas include glioblastoma, the most common adult brain tumor, and the highly aggressive pediatric malignancy, diffuse intrinsic pontine glioma (DIPG), among others. Michelle and her team showed that 5-10% of glioma cells within each tumor receive synaptic signals, and about 40% exhibit prolonged potassium-evoked currents that are amplified via gap junctions. Boosting electrical signals into tumors accelerated their growth. The researchers also noticed that healthy neurons near tumors tend to be hyperexcitable, which could explain why many HGG patients suffer seizures. Experiments demonstrated that interrupting these signals with an existing anti-epilepsy drug greatly reduced the growth of human gliomas in mice. The team is investigating whether blocking electrical signaling within tumors could help people with HGGs.

Michelle Monje
Ludwig Stanford

SIMPLE NEEDS

When T cells detect a pathogen or a cancer cell, they switch from a quiescent state into an active one. The process can be metabolically taxing and depends on the availability of several nutrients, including glucose and the amino acid glutamine. A collaborative effort by Ludwig Harvard's Marcia Haigis, Arlene Sharpe and their colleagues has now identified a new amino acid that is necessary for promoting this cell state transition. In a study published in September in *Cell Reports*, Marcia's team found that the activation of naïve T cells, and the reactivation of memory T cells, is dependent on the import of a simple amino acid, alanine. This amino acid is manufactured rather easily by most cells—but not by T cells, which produce low levels of an enzyme essential to alanine biosynthesis and are thus obliged to import the amino acid from extracellular nutrient pools. Radioisotope tracing revealed that all the alanine brought in by T cells is quickly directed toward protein synthesis rather than being broken down for other metabolic purposes. Tumors are known to deprive T cells of many vital nutrients, like glucose, to suppress immune surveillance. It remains to be seen whether tumor-mediated alanine depletion too aids immune escape.

Marcia Haigis
Ludwig Harvard

Arlene Sharpe
Ludwig Harvard

PRIME AND BOOST

Vaccitech Oncology Limited (VOLT), a strategic collaboration of the Ludwig Institute for Cancer Research and the biotech Vaccitech, entered into a clinical partnership with Cancer Research UK (CRUK) to develop VOLT's VTP-600 immunotherapy as a treatment option for patients with non-small cell lung cancer (NSCLC). In 2019, lung cancer accounted for 142,670 deaths in the US and approximately 85% of those cases are expected to be NSCLC. New treatments are urgently needed, as the overall 5-year survival rate for lung cancer is 17%. The vaccine, carrying two antigens discovered by Ludwig scientists—MAGE-A3 and NY-ESO-1—on a Vaccitech delivery platform is designed to first prime and then boost a T cell attack on cancer cells. The Phase I/IIa trial sponsored and managed by CRUK will test the vaccine in combination with standard first-line chemotherapy and anti-PD-1 immunotherapy and assess its ability to provoke a safe and effective anti-cancer immune response in approximately 80 patients with NSCLC. Patients will be screened for their tumors' expression of MAGE-A3 and NY-ESO-1 to maximize the likelihood of benefit. The trial is slated to begin later this year across multiple clinical sites in the UK.

ROBERT WEINBERG

DIRECTOR OF THE LUDWIG CENTER FOR MOLECULAR ONCOLOGY AT MIT

How would you summarize your research in a tweet?
Trying to understand the basic mechanistic principles of how human cancers arise.

What central questions drive your lab's research?
How do disseminated cancer cells, once they leave a primary tumor and land in a distant tissue, figure out how to make a living? They face a difficult challenge and only rarely succeed. How they do it is still a bit obscure, and my lab group has been focusing a lot of energy on that particular question.

What was the greatest impact of your discoveries of the first human cancer-causing gene and the first tumor suppressor gene?
We began studying viral oncogenes in 1977, which led to the discovery of the first cellular oncogene Ras in 1979. I like to refer to this discovery as "an earned run" because of the work involved in identifying, isolating and cloning it. However, the subsequent discovery of the first tumor suppressor gene Rb in 1986 was "an unearned run," since it fell into my lap thanks to work of post-docs Stephen Friend and René Bernards. These two discoveries laid out a template, or blueprint, for how one could, in principle, understand human cancer pathogenesis through the actions of either hyperactive oncogenes or inactive tumor suppressor genes. Until then these mechanisms were simply matters of speculation, that is, there was no direct proof that one could really use the tools of molecular biology that were then rapidly developing in order to reveal the mechanisms that allowed activated oncogenes or inactivated tumor suppressor genes to push forward the growth of genetically altered cells.

Why aren't malignant tumors just content to stay put as they grow and get bigger?
When tumors begin in different sites in the body, the question is—are they intent on spreading? To my mind, metastatic dissemination of cancer cells from a primary tumor site to distant sites in the body is an accident that happens when the carcinoma cells increasingly create an inflamed tissue environment around them that, in turn, provokes these cells to begin to invade and ultimately to disseminate physically to distant tissues. Once they are there, they confront the problem of how they are going to make a living in these distant tissues. That remains one aspect of this problem that is essentially unsolved. In contrast, the question of how mechanistically cancer cells move from a primary tumor to a distant tissue, at least in the case of carcinomas, is basically solved in the context of understanding the complex cell-biological program often called the EMT—epithelial-to-mesenchymal transition—that imparts to carcinoma cells virtually all of the attributes that we ascribe to the cancer cells of high-grade malignancies.

> "I think the field as a whole is a bit stuck in the mud. We don't really know how to break through all the obstacles that are preventing profound improvements in the way we treat cancer."

How did the writing of *The Hallmarks of Cancer* come about?
Writing *The Hallmarks of Cancer* was an accident. Doug Hanahan and I were playing hooky one afternoon from a

conference in Hawaii, and we were walking down the mouth of a volcano when we began to talk about the fact that, unlike the laws of physics, the laws of cancer biology were quite messy and not clearly conceptualized. Both he and I were products of an MIT education, so we had great faith in the powers of fundamental principles to explain all kinds of phenomena. And indeed, we perceived cancer research to be just a collection of phenomena. We began to discuss whether we could articulate a set of underlying principles that might enable people to undertake comparisons between different kinds of tumors. We wrote the review in 1999 fully expecting that, like most reviews, it would sink like a stone thrown into a quiet pond. It appeared in the first issue of *Cell* in January 2000. To our surprise, indeed total astonishment, it turned out to be very helpful in allowing people to understand the complexities of cancer in terms of a relatively small number of underlying mechanistic principles.

How did *The Hallmarks of Cancer* influence your own personal research?
I was able to create a dichotomy in my thinking about cancer progression. On the one hand, what are the principles responsible for creation of the cells forming a primary tumor? On the other, what principles or mechanisms are responsible for its metastatic spread? That dichotomy has strongly influenced my thinking, because I've often argued that the formation of the primary tumor is largely the responsibility of genetic mutations that strike the genomes of cancer cells, whereas the metastatic spread that comes after is largely the consequence of cell-biological programs that cannot be understood by sequencing the genomes of cancer cells.

Who are some of the mentors who have supported you throughout your career?
Arguably the person who supported me the most during my career was David Baltimore. I knew him when he

was a graduate student at MIT in 1963-64, and it was he who helped make the necessary connections so that I could get a postdoc at the Salk Institute in 1970-71. It was he who whispered in the ear of Salvador Luria, who was the founder and first director of the MIT Center for Cancer Research, to hire me as a junior faculty member. David was the founding director of the Whitehead Institute and in 1982 brought me on board. So, in many respects, much of what I am is due to the fact that I had continuing support and inspiration from him.

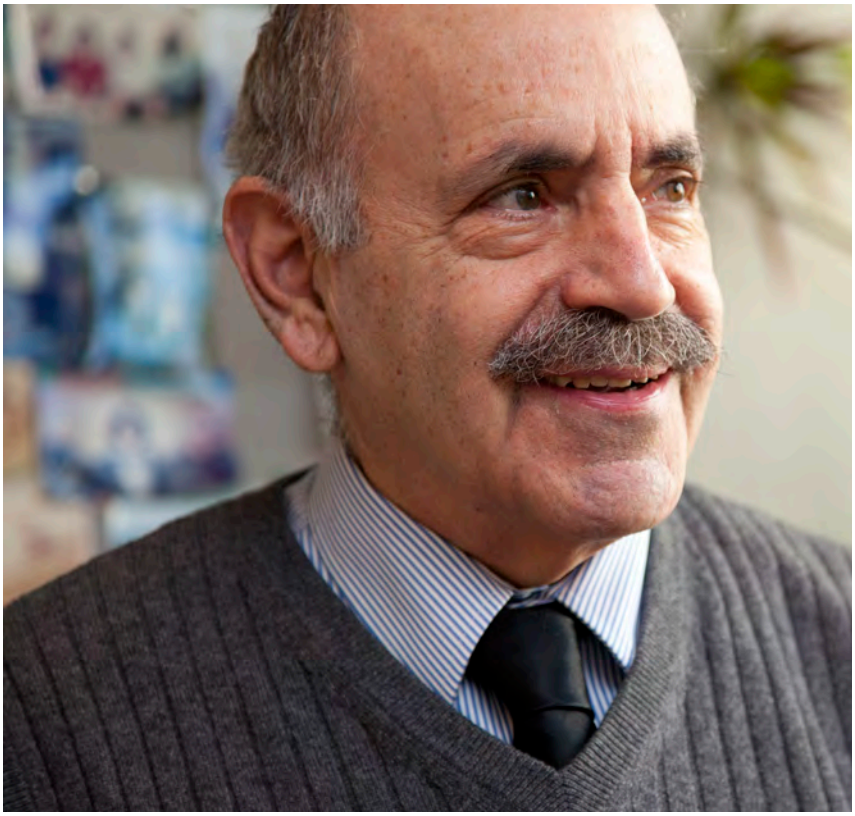
How do you mentor and support the next generation of scientists?
Teaching and mentoring are as important as doing research. At MIT, every faculty member in the biology department is required to teach. Early on, I realized that training and mentorship in my lab are as important as the results that we produce from our work. They're what ensure that cancer research continues to move forward. And so, for three or four decades I've actually been very active in mentoring the people in my lab. I have also been very active both at MIT and in the greater Boston community in organizing a series of meetings where young people have a chance to see how cancer research is conducted and learn how to thrive as independent investigators. In my own case, the explicitly stated goal of my lab is that the trainees who pass through the lab should be able to stand on their own two feet and launch their own independent research careers after they have left my lab. If they are unsuccessful in doing so, I regard that as a failure on my part.

In this climate, where funding is more challenging for scientists, how do you encourage your trainees to pursue a career in science?
Whether they go into academia, biotech, big pharma or the law, I tell them that my only job as their mentor is to tune-up their brain. Once it's tuned-up, I encourage them to figure out what they're best at and what they enjoy doing most. So, I would say it's more of a positive message rather than one that bewails the plight of research funding.

What is the best piece of advice you have ever received from a mentor?
It came from my father who said to me on a number of occasions—it's all right to be successful, but don't be too visible. This stemmed from his having fled Germany in the 1930s where, if you were too successful or too visible, one night the guys in the brown shirts would come knocking on your door. That advice has remained very much at the front of my mind. I've tried not to be too self-aggrandizing because, as I've often told my people, "success breeds envy and resentment."

What are the greatest challenges facing the cancer research field?
I envision two big challenges on the horizon. The first is how to analyze complex data sets and glean some simple biological take-home lessons from them. Many claim that bioinformatics and artificial intelligence will solve the entire problem, but I remain very skeptical. The second is how to integrate the behavior of the genetic alterations

"Early on, I realized that training and mentorship in my lab are as important as the results that we produce from our work."



ensconced in the cancer cell genomes with the transcriptomes—the repertoire of genes that are being transcribed in that particular cell. We don't have a very clear picture about how the genetic changes and the epigenetic programs interact with one another to ultimately create the final biological phenotype of the cancer cell. Understanding and solving those two problems is critical to the future success of cancer research.

What do you think are the greatest opportunities in cancer research today?
I think the field as a whole is a bit stuck in the mud. We don't really know how to break through all the obstacles that are preventing profound improvements in the way we treat cancer. Immunotherapy seems very attractive, and it might represent an enormous opportunity, if only we could figure out how to make it work in most kinds of tumors. At present, we don't really know how to do that. So, I would argue that the greatest opportunity is to improve the efficacy of checkpoint immunotherapy so that it can be widely applicable to successfully treat a whole series of solid human tumors.

What are the areas of cancer research that hold the greatest promise for cancer patients?
I'm optimistic about four areas of opportunity. First is how to use the various existing treatments in combination. Second, how to potentiate the efficacy of checkpoint immunotherapy, which at present is quite limited in terms of its widespread applicability. Third, how to harness our insights into the metabolic aberrations of cancer cells in order to selectively hit them and, fourth, being able to switch aggressive carcinoma cells into a less aggressive state by eradicating or differentiating aggressive, undifferentiated tumor-initiating carcinoma cells.

If you had the resources and the power to eradicate any world problem other than cancer, what would you choose to solve?
Climate change. It's the greatest challenge that threatens all of humanity. It's gravity and breadth overshadow any other human problem.

If we fail to address this problem successfully, there will be catastrophic consequences for our children and grandchildren.

Who are the scientists living or dead that you admire most?
The Nobel Laureate Dan Nathan at Johns Hopkins. He was a man of great success, great intellect and yet someone who was extraordinarily humble and self-effacing. For me, emulating Dan Nathan has always been a goal I've aspired to.

What moment in history would you have most liked to have witnessed?
The moment Hitler committed suicide in the Berlin bunker.

If you could write the title of the story of your life, what would it be?
One Step After Another: A Whole Concatenation of Accidents.

When you reflect on your career, what are you most proud of?
The 1979 paper in which we demonstrated that the DNA of a chemically transformed cell actually carried genetic information that could convert a normal cell into a cancer cell.

What were the values that were espoused in your household growing up?
Growing up in a family of European refugees, it was clear that the characteristics my parents and grandparents valued most in a person were, firstly, intelligence and, secondly, a sense of humor. If a person wasn't too bright, that person could make up for it if they had a good sense of humor! Never do I ever recall their mentioning financial or material success in terms of how they valued other human beings.

> "Immunotherapy seems very attractive, and it might represent an enormous opportunity, if only we could figure out how to make it work in most kinds of tumors. At present, we don't really know how to do that."

If you had not become a cancer researcher, what other career paths do you think you might have taken?
A carpenter. There is something very satisfying about working with my hands. Starting in 1976, my wife and I built our cabin in New Hampshire and over the years added two more wings and a porch. In the summer, I'm a fanatic gardener and relish repairing things. Outside of the lab, I like to ponder simple problems like what size bolt will I use to attach a joist to a beam, and I'm endlessly fascinated by how things get connected and the design of complex structures. To this day, when we go by a construction site, I insist that my family stop so I can study what the workers are doing.

What is the best piece of advice you ever received from a mentor?

In research, 90% of your days will be spent struggling with so-called "negative results" that go against your original hypothesis. However, if you can be more flexible with your ideas, as long as you do the right controls, you will be able to take these results positively to advance knowledge.

PEDRO MOURA-ALVES
Ludwig Oxford

As I was finishing graduate research, my thesis advisor, Hal Weintraub, told me that it really didn't matter what particular problem I worked on next, so long as important questions were experimentally tractable. That advice has always influenced my work, from academic to industrial settings, and back again at Ludwig Cancer Research.

ROBERT DAVIS
Ludwig San Diego

Prioritize the challenging experiment that will definitively disprove your hypothesis. You might be tempted to spend time on easier experiments that may not directly answer your question, but ultimately this delays the inevitable.

REBEKAH BROOKS
Ludwig Wistar

The best piece of advice I have received from a mentor was to find my own path as a scientist and to not compare it with others. Being a scientist means to apply the knowledge and inspiration you get from others and transform it into something personal and unique.

ELISABETTA CRIBIOLI
Ludwig Lausanne

Required reading

Ludwig Brussels

Cancer Immunology Research
2019 December 5
[Epub ahead of print]

Tryptophan 2,3-dioxygenase expression identified in human hepatocellular carcinoma cells and in intratumoral pericytes of most cancers.

Hoffmann D, Dvorakova T, Stroobant V, Bouzin C, Daumerie A, Solvay M, Klaessens S, Letellier MC, Renauld JC, van Baren N, Lelotte J, Marbaix E, Van den Eynde BJ.

Ludwig Harvard

Proceedings of the National Academy of Sciences USA
2019 December 26
[Epub ahead of print]

CCR2 inhibition reduces tumor myeloid cells and unmasks a checkpoint inhibitor effect to slow progression of resistant murine gliomas.

Flores-Toro JA, Luo D, Gopinath A, Sarkisian MR, Campbell JJ, Charo IF, Singh R, Schall TJ, Datta M, Jain RK, Mitchell DA, Harrison JK.

Cell Systems
2019 December 2
[Epub ahead of print]

Torin2 exploits replication and checkpoint vulnerabilities to cause death of PI3K-activated triple-negative breast cancer cells.

Chopra SS, Jenney A, Palmer A, Niepel M, Chung M, Mills C, Sivakumaren SC, Liu Q, Chen JY, Yapp C, Asara JM, Gray NS, Sorger PK.

Nature
2019 October 16
[Epub ahead of print]

Altered chromosomal topology drives oncogenic programs in SDH-deficient GISTs.

Flavahan WA, Drier Y, Johnstone SE, Hemming ML, Tarjan DR, Hegazi E, Shareef SJ, Javed NM, Raut CP, Eschle BK, Gokhale PC, Hornick JL, Sicinska ET, Demetri GD, Bernstein BE.

Cell Reports
2019 September 17

T cell activation depends on extracellular alanine.

Ron-Harel N, Ghergurovich JM, Notarangelo G, LaFleur MW, Tsubosaka Y, Sharpe AH, Rabinowitz JD, Haigis MC.

Ludwig Institute

Cell Reports
2019 November 12

Misregulation of Drosophila Myc disrupts circadian behavior and metabolism.

Hsieh AL, Zheng X, Yue Z, Stine ZE, Mancuso A, Rhoades SD, Brooks R, Weljie AM, Eisenman RN, Sehgal A, Dang CV.

Ludwig Johns Hopkins

Proceedings of the National Academy of Sciences USA
2019 Sep 23
[Epub ahead of print]

Cell division rates decrease with age, providing a potential explanation for the age-dependent deceleration in cancer incidence.

Tomasetti C, Poling J, Roberts NJ, London NR Jr, Pittman ME, Haffner MC, Rizzo A, Baras A, Karim B, Kim A, Heaphy CM, Meeker AK, Hruban RH, Iacobuzio-Donahue CA, Vogelstein B.

Ludwig Lausanne

Nature Chemical Biology
2019 December 2
[Epub ahead of print]

Computational design of G protein-coupled receptor allosteric signal transductions.

Chen KM, Keri D, Barth P.

Molecular Therapy Oncolytics
2019 December 24

MIR-155 overexpression in OT-1 CD8+ T cells improves anti-tumour activity against low affinity tumor antigen.

Monnot GC, Martinez-Usatorre A, Lanitis E, Lopes SF, Cheng W-C, Ho P-C, Irving M, Coukos G, Donda A, Romero P.

Journal of ImmunoTherapy Cancer
2019 November 18

Immunopeptidomics of colorectal cancer organoids reveals a sparse HLA class I neoantigen landscape and no increase in neoantigens with interferon or MEK-inhibitor treatment.

Newey A, Griffiths B, Michaux J, Pak HS, Stevenson BJ, Woolston A, Semiannikova M, Spain G, Barber LJ, Matthews N, Rao S, Watkins D, Chau I, Coukos G, Racle J, Gfeller D, Starling N, Cunningham D, Bassani-Sternberg M, Gerlinger M.

Nature Biotechnology
2019 October 14
[Epub ahead of print]

Robust prediction of HLA class II epitopes by deep motif deconvolution of immunopeptidomes.

Racle J, Michaux J, Rockinger GA, Arnaud M, Bobisse S, Chong C, Guillaume P, Coukos G, Harari A, Jandus C, Bassani-Sternberg M, Gfeller D.

Ludwig MSK

Lancet Oncology
2019 December 20
[Epub ahead of print]

Transcriptomic signatures related to the obesity paradox in patients with clear cell renal cell carcinoma: a cohort study.

Sanchez A, Furberg H, Kuo F, Vuong L, Ged Y, Patil S, Ostrovnaya I, Petruzella S, Reising A, Patel P, Mano R, Coleman J, Russo P, Liu CH, Dannenberg AJ, Chan TA, Motzer R, Voss MH, Hakimi AA.

Required reading

Cell
2019 October 24
[Epub ahead of print]

Transcriptional basis of mouse and human dendritic cell heterogeneity.

Brown CC, Gudjonson H, Pritykin Y, Deep D, Lavallée VP, Mendoza A, Fromme R, Mazutis L, Ariyan C, Leslie C, Pe'er D, Rudensky AY.

Ludwig Oxford

Proceedings of the National Academy of Sciences USA
2020 January 21
[Epub ahead of print]

CagA-ASPP2 complex mediates loss of cell polarity and favors H. pylori colonization of human gastric organoids.

Buti L, Ruiz-Puig C, Sangberg D, Leissing TM, Brewer RC, Owen RP, Sgromo B, Royer C, Ebner D, Lu X.

Scientific Reports
2019 December 10

Co-incidence of RCC-susceptibility polymorphisms with HIF cis-acting sequences supports a pathway tuning model of cancer.

Schmid V, Lafleur VN, Lombardi O, Li R, Salama R, Colli L, Choudhry H, Chanock S, Ratcliffe PJ, Mole DR.

Ludwig San Diego

Nature
2019 November 20
[Epub ahead of print]

Circular ecDNA promotes accessible chromatin and high oncogene expression.

Wu S, Turner KM, Nguyen N, Raviram R, Erb M, Santini J, Luebeck J, Rajkumar U, Diao Y, Li B, Zhang W, Jameson N, Corces MR, Granja JM, Chen X, Coruh C, Abnousi A, Houston J, Ye Z, Hu R, Yu M, Kim H, Law JA, Verhaak RGW, Hu M, Furnari FB, Chang HY, Ren B, Bafna V, Mischel PS.

Ludwig Stanford

Nature
2019 December 4
[Epub ahead of print]

c-Jun overexpression in CAR T cells induces exhaustion resistance.

Lynn RC, Weber EW, Sotillo E, Gennert D, Xu P, Good Z, Anbunathan H, Lattin J, Jones R, Tieu V, Nagaraja S, Granja J, de Bourcy CFA, Majzner R, Satpathy AT, Quake SR, Monje M, Chang HY, Mackall CL.

Science Translational Medicine
2019 November 20

Therapeutic strategies for diffuse midline glioma from high-throughput combination drug screening.

Lin GL, Wilson KM, Ceribelli M, Stanton BZ, Woo PJ, Kreimer S, Qin EY, Zhang X, Lennon J, Nagaraja S, Morris PJ, Quezada M, Gillespie SM, Duveau DY, Michalowski AM, Shinn P, Guha R, Ferrer M, Klumpp-Thomas C, Michael S, McKnight C, Minhas P, Itkin Z, Raabe EH, Chen L, Ghanem R, Geraghty AC, Ni L, Andreasson KI, Vitanza NA, Warren KE, Thomas CJ, Monje M.

Nature
2019 September 18
[Epub ahead of print]

Electrical and synaptic integration of glioma into neural circuits.

Venkatesh HS, Morishita W, Geraghty AC, Silverbush D, Gillespie SM, Arzt M, Tam LT, Espenel C, Ponnuswami A, Ni L, Woo PJ, Taylor KR, Agarwal A, Regev A, Brang D, Vogel H, Hervey-Jumper S, Bergles DE, Suvà ML, Malenka RC, Monje M.

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