LUDWIG LINK

JUNE 2018

IN THIS ISSUE

LUDWIG CANCER RESEARCH

LIFE-CHANGING SCIENCE

LUDWIG LINK
ludwigcancerresearch.org JUNE 2018

LETTER

If you've been wondering what your colleagues have been up to these past few months, take a gander through this spring issue of Ludwig Link. Turns out, it's quite a lot. You'll read in here about the anticancer power of nanoflowers, how surgery can fire up dispersed breast cancer cells and how an individualized cancer vaccine extended the lives of women with advanced ovarian cancer. Plus, as usual, much, much more.

Our interview in this issue is with Ludwig Lausanne's Johanna Joyce, our newest Member and a gifted cancer immunologist who has transformed our understanding of the tumor microenvironment.

It's hard to imagine a life before social media, even if the technologies have only been with us for about a decade. So we asked our researchers to weigh in on what value networks like Facebook and Twitter have to science and scientists. Their answers are on page 24.

We wish you all a wonderful summer!

Sincerely,

Rachel Reinhardt
Vice President for Communications

On the cover: From left, Ludwig Oxford's Benjamin Schuster-Böckler, Skirmantas Kriaucionis and Chunxiao Song

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Don Cleveland

Ludwig San Diego

FOR SILENCING SICKNESS

Ludwig San Diego's Don Cleveland won the prestigious Breakthrough Prize in Life Sciences in December for his work on the molecular mechanisms of inherited neurodegenerative disorders such as ALS, or Lou Gehrig's disease, and Huntington's disease. His groundbreaking accomplishments date back to the 1970s, when he identified the abnormal protein tau, which accumulates in the brains of patients with Alzheimer's disease and chronic brain injury. Don has devised designer DNA drugs for diseases of the brain and nervous system, and collaborated with a biotech company to develop a drug for Huntington's disease that has safely lowered the disease-causing mutant protein. A large efficacy trial is slated to start this year. Some 400 children with spinal muscular atrophy, an inherited muscle wasting condition that was once invariably fatal, have been treated this year with a DNA drug stemming from his research, allowing many who were previously immobilized to walk. Similar designer DNA drugs are currently in clinical trials for the treatment of ALS, Huntington's disease and Alzheimer's disease. Click here to watch the award program. Don's segment begins at 1:01.

FOR METABOLIC INSIGHT

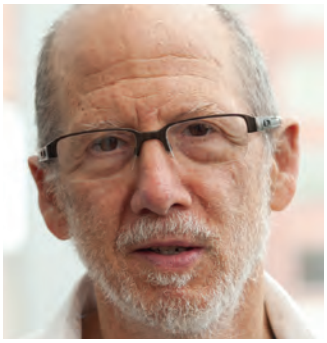
Ludwig's Scientific Director Chi Van Dang was elected Fellow of the American Association for Cancer Research (AACR) Academy in April. A physician and influential researcher, Chi is best known for defining the complex functions of MYC—a gene whose mutation or aberrant expression is associated with many types of cancer—and its central role in rewiring the cancer cell's metabolism to support uncontrolled proliferation. This body of work, which explained a hallmark of tumor metabolism known as the "Warburg effect," bolstered the hypothesis that cancer cells can become addicted to their rewired metabolic pathways and dependent on certain nutrients. It also showed that disrupting those pathways could be a powerful approach to treating cancer. His lab has more recently integrated its work on cancer metabolism with an ongoing exploration of the circadian clock, or the molecular signals that govern cellular responses to the day-night cycle. Chi's team has shown how the Myc protein can disrupt that clock, undoing the restraints that prompt cells to "go" during the day and replenish and repair themselves at night. Chi's studies have led to the design of many new cancer therapies that are in various stages of development.

Chi Van Dang

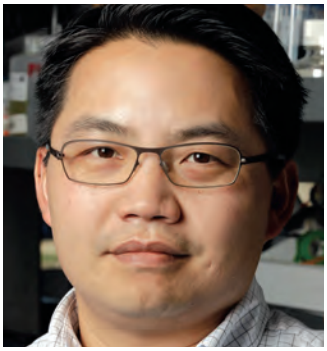
Ludwig Institute

FOR ADVANCING PERSONALIZED ONCOLOGY

Ludwig Johns Hopkins' Bert Vogelstein was one of three US cancer researchers awarded the Dan David Prize in February for their pioneering discoveries in the field of personalized medicine. Bert was selected for his seminal contributions to our understanding of how genetic changes drive human cancers. Bert and his team have identified more than 20 of the most commonly altered genes that drive tumors of multiple types and shown how these genetic alterations accumulate during tumor progression. Based on these findings, he and his colleagues created genetic tests for hereditary colon cancers. They also pioneered the use of genetic alterations as exquisitely specific biomarkers for disease. This work has led to "liquid biopsies" and the first FDA-approved screening test for sporadic cancers based on genetics. The US $1,000,000 prize will be shared among the three scientists, who will donate 10% of their awards to postgraduate students to help cultivate a new generation of scholars.



Bert Vogelstein
Ludwig Johns Hopkins



Howard Chang
Ludwig Stanford

FOR INVENTIVENESS AND DISCOVERY

Howard Chang, the Virginia and D. K. Ludwig Professor of Cancer Genomics at Stanford, received the National Academy of Sciences (NAS) award in molecular biology for his discovery of long noncoding (lnc) RNAs, a sprawling family of RNA molecules that control gene activity throughout the genome. lncRNAs, which number in the thousands, play critical roles in everything from embryonic development and aging to cancer and its metastasis. He and his team are exploring the mechanisms by which these ubiquitous RNA molecules regulate genomic expression, and have already shown distinct ways in which certain lncRNAs interact with chromatin—DNA and its protein scaffolding—to silence and activate genes. Howard's lab has also developed ingenious new methods for the wholesale functional mapping of the genome. The NAS noted that a method his lab developed has "improved the ability to map active DNA elements by 1 million-fold in sensitivity and 100-fold in speed." Howard received a medal and a $25,000 prize from the NAS on April 29 in Washington, D.C.

Stefan Constantinescu
Ludwig Brussels

FOR BATTLING BLOOD CANCER

Ludwig Brussels' Stefan Constantinescu has won one of two Quinquennial Awards of the Federal Government of Belgium for his research into the molecular bases of blood cancers, especially chronic myeloproliferative neoplasms (MPNs). In these blood cancers the body makes too many red blood cells, platelets, or white blood cells. Stefan co-discovered the JAK2 V617F mutation, which is a common driver of MPNs. He also identified activating mutations in Tpo receptor (TpoR) in MPNs that do not involve JAK2 V617F. JAK2 and TpoR molecular testing is now an essential part of the diagnosis of MPNs, and JAK2 inhibitors are in the clinic. In addition, Stefan identified the first activating TYK2 and JAK1 mutations, which are involved in T-cell acute lymphoblastic leukemia. The prizes have been awarded since 1859 by a jury representing the Royal Dutch- and French-speaking Academies of Medicine of Belgium and are awarded to Belgian researchers.

FOR ILLUMINATING CELL DIVISION

Ludwig San Diego's Karen Oegema and Arshad Desai were named Fellows of the American Society for Cell Biology (ASCB) for their "meritorious efforts to advance cell biology and its applications and for their service to ASCB." Karen's research applies advanced microscopy and functional genomics to probe the gene networks and molecular mechanisms underlying cell division and embryonic development, which are also sometimes aberrant in cancer cells. Arshad's lab explores how the replicated genome is accurately parceled out to daughter cells during cell division—a tightly orchestrated process often compromised in cancer cells. The new ASCB Fellows were recognized at the 2017 ASCB|EMBO Meeting in Philadelphia in December at a special reception and awards ceremony.

Karen Oegema
Ludwig San Diego

Arshad Desai
Ludwig San Diego

FOR GLOWING SCIENCE

Ludwig Chicago Co-director Ralph Weichselbaum received the David A. Karnofsky Memorial Award and Lecture of The American Society of Clinical Oncology (ASCO) at the Society's annual meeting in June. An expert in radiotherapy, Ralph has devoted his career to exploring how radiotherapy kills cancer cells and developing new approaches to boost those effects. He is probably best known for advancing, in partnership with Ludwig Board Member Samuel Hellman (see page 7), the idea that cancers can exist in an intermediate state—named oligometastasis—between local and systemic disease. Such cancers, they proposed, can be identified by their clinical presentation and molecular traits, and can in many cases be cured by surgery or radiotherapy alone. More recently, Ralph has explored how radiotherapy engages the immune system to destroy tumors, the mechanisms by which tumors resist such attack—and how such knowledge might be exploited to improve both radiotherapy and immunotherapy (see pages 10 and 16).

Ralph Weichselbaum
Ludwig Chicago

Alexander Rudensky
Ludwig MSK

FOR TREG TRIUMPHS

Ludwig MSK Director Alexander Rudensky received the US $100,000 Vilcek Prize in biomedical science for his decades-long study of regulatory T cells (Tregs) and their part in a wide array of bodily functions and dysfunctions. Alexander's laboratory played a central role in the discovery and characterization of these T cells, which are essential to preventing autoimmunity and tamping down protective immune responses. He and his colleagues, together with two other groups, discovered that Treg cells are defined by the expression of a transcription factor (a master regulator of gene expression) named FOXP3, and went on to unravel the molecular mechanisms essential to everything from their maintenance of identity to their control of killer T cells to their influence on cancer progression and wound healing. The prize is sponsored by the Vilcek Foundation, which was established in 2000 by Czechoslovakian immigrants Jan and Marica Vilcek to raise awareness of the contributions immigrants make to American life and science.

LEAVING LUDWIG'S BOARD

Samuel Hellman will retire from the Ludwig Board this June, having served on the body since 2009. Prior to his tenure on the board, Sam spent 14 years on Ludwig's Scientific Advisory Committee. We thank him for his invaluable service to Ludwig. Sam has packed a couple of lifetimes of work into his career, but he is perhaps best known for his studies on the natural history of breast cancer and for pioneering the use of lumpectomy and radiation as an alternative to the then primary treatment of radical mastectomy. This breakthrough helped physicians avoid removing the breast and muscles of the chest wall, a measure that had extremely deleterious cosmetic and functional effects. He also conceived the idea of oligometastasis and fleshed it out in (ongoing) studies in the clinic and the laboratory in collaboration with Ludwig Chicago's Ralph Weichselbaum (see feature on page 6). Sam is renowned as an educator and leader, having been physician-in-chief at Memorial Sloan Kettering, Dean of the Pritzker School of Medicine at the University of Chicago and chairman of radiation therapy at Harvard Medical School. He has served as president of the American Society for Therapeutic Radiology and Oncology and the renowned American Society of Clinical Oncology. A co-editor of seven editions of the standard textbook, *Cancer: Principles and Practice of Oncology*, Sam is also a prolific author in his own right. His writings were compiled in a book published last year, *Learning While Caring: Reflections on a Half-Century of Cancer Practice, Research, Education and Ethics*. In it he suggests you should "love and value what you do." Sam, it appears, is the sort of man who follows his own advice.

Samuel Hellman
Ludwig Institute

Thomas Baenninger
Ludwig Institute

OUR NEW CFO

Thomas Baenninger was appointed CFO, succeeding Richard Walker, who stepped down from that post earlier this year. Thomas joined Ludwig in September 2017 as Deputy CFO and assumed CFO responsibilities in February. He has two decades of experience managing the financial operations of major corporations and joined Ludwig from the publicly traded Swiss industrial engineering and manufacturing firm Sulzer Ltd. As post-merger integration manager at Sulzer, Thomas led the integration of GEKA into Sulzer's business after its acquisition in 2016. Prior to that, he served for a dozen years as CFO of the global Chemtech division of Sulzer, leading a team of 20 professionals responsible for the division's financial administration and reporting. Before joining Sulzer, Thomas served for six years as the CFO and Chief Information Officer of Bucher Automotive, a division of the Swiss equipment and machine manufacturer Bucher Industries.

SWEET SUCCESS

Each year, some 300 children in the US are diagnosed with diffuse intrinsic pontine gliomas (DIPGs), aggressive tumors that develop at the base of the brain. The cancer is incurable and typically causes death within 10 months of diagnosis. Now, a study led by Ludwig Stanford's Crystal Mackall and Michelle Monje and published in May in *Nature Medicine* reports the first near-eradication of DIPG in mouse models. The researchers found that a mutation that drives DIPG cell proliferation, known as H3K27M, is responsible for high levels of a complex sugar called GD2 on DIPG cells. Crystal had already engineered chimeric antigen-receptor T (CAR-T) to target GD2, which is a CAR-T target in other cancers as well. The team tested them in mouse models of DIPG developed in Michelle's lab and found that they demolished DIPG tumors, leaving just a smattering of cancer cells that did not express GD2 (which could, they note, seed a relapse). An area of concern was the expected brain inflammation in the vicinity of the brainstem, which caused trouble for some treated mice. Still, the team hopes to create safeguards to minimize and manage this risk and move their CAR-T immunotherapy into human clinical trials.

Crystal Mackall
Ludwig Stanford

Michelle Monje
Ludwig Stanford

Bob Weinberg
Ludwig MIT

ANOTHER WIN FOR ASPIRIN?

One in four women who undergo a lumpectomy or mastectomy will experience a cancer relapse, apparently because the surgery itself provokes the outgrowth of disseminated cancer cells that had until then been kept in check, possibly by the immune system. To explore how this happens, a team led by Ludwig MIT Director Bob Weinberg created a mouse in which the immune system's T cells suppressed cancer cells implanted in the animals. They then simulated surgeries at sites far from the injection points and found that tumor size and incidence grew dramatically in these mice. Their analysis, published in an April issue of *Science Translational Medicine*, indicates that wound-healing following surgery elevates the systemic incidence of immune cells called inflammatory monocytes. These can mature into tumor-associated macrophages, which suppress the T cell's anti-tumor responses. Even more intriguing is their finding that when the mice were treated with an aspirin-like nonsteroidal anti-inflammatory drug (meloxicam) during or after surgery, the post-operative mice developed fewer and smaller tumors but healed as well as ever. The findings may be preliminary, and obtained in mice, but aspirin and its kin certainly are building quite the anti-cancer rep.

Jedd Wolchok
Ludwig MSK

Dmitriy Zamarin
Ludwig MSK

CANCER HACK

Oncolytic virotherapy (OV) deploys naturally-occurring and engineered viruses to destroy tumors. The viruses selectively infect cancer cells, destroying them as the virions break out to infect other cancer cells. Even better, this reveals hidden cancer antigens that can activate an anti-tumor immune response. OV can, however, also activate immune signaling in tumors that mutes such effects. A team led by Ludwig MSK's Jedd Wolchok and Dmitriy Zamarin analyzed human tumor cultures and mouse tumor models treated with a Newcastle disease virus (NDV)—which ordinarily infects only birds—to identify drug targets that might overcome such immunosuppression. Their paper, in an April issue of the *Journal of Clinical Investigation*, reports that infection with NDV led to an increase of PD-L1 expression on tumor cells and tumor-infiltrating immune cells. (PD-L1 suppresses the killer T cell attack.) Treating a tumor with NDV in combination with systemic PD-1 or PD-L1 blockade resulted in the rejection of both the treated and distant tumors. The paper also identifies a variety of pathways that might be targeted to overcome OV-induced immune suppression. The findings have implications for how immunotherapies are selected and delivered to enhance OV.

JAK-ING DOWN RESISTANCE

Non-small cell lung cancer (NSCLC), the most common and deadliest form of lung malignancies, tends to quickly develop resistance to DNA-damaging chemo and radiotherapy. And though immune checkpoint blockade has significantly improved NSCLC treatment, not all patients respond equally well to these immunotherapies. Now, it appears both obstacles might be overcome with one selective inhibitor of the signaling protein JAK2. A team led by Ludwig Chicago's Nikolai Khodarev and Ralph Weichselbaum reported in an April issue of *Molecular Cancer Therapeutics* that this drug, SAR302503, kills cells that have developed resistance to DNA damaging therapies. Sensitivity to this inhibitor can be predicted by patterns of gene expression induced by ceaseless interferon (IFN) signaling—including one that Nikolai and Ralph have previously shown to be associated with such resistance in a variety of cancer cell lines. Further, PD-L1 expression, which shields tumor cells from immune attack, is also induced in NSCLC cells by aberrant IFN signaling—and shut down by SAR. This suggests JAK2 inhibitors may be useful both as monotherapies and as drugs given in combination with checkpoint inhibitors to NSCLC patients who have stopped responding to standard therapies.

Nikolai Khodarev
Ludwig Chicago

Ralph Weichselbaum
Ludwig Chicago

Stephen Elledge
Ludwig Harvard

WHY WHERE MATTERS

A study led by Ludwig Harvard's Stephen Elledge and published in *Cell* in April shows that tissue type plays an outsize role in cancer genetics and should be taken into consideration when devising therapies. The paper reports a trove of hundreds of cancer-driving genes that may not be detected by genome sequencing, and reveals that different tissue types have differing sensitivities to these oncogenes: A set that drives, say, pancreatic malignancies may be far less malignant in breast tissue. The researchers barcoded some 30,000 genes and put one of them into each of a set of cells, which they then grew in the same container. The barcodes revealed which genes were the fiercer drivers of proliferation in breast cells, pancreatic cells and fibroblasts—cells that make connective tissue. Stephen and colleagues found that 10% of the genes regulate proliferation and identified previously unknown copy number changes to 254 genes (147 amplifications, 107 deletions) that are of relevance to cancer. But what surprised most was the stark diversity of responses to pro-growth genes in each tissue type. The finding suggests that selecting therapies based on specific and common cancer drivers might be more complicated than it appears.

Rakesh Jain
Ludwig Harvard

DANGEROUS FAT

Ludwig Harvard investigator Rakesh Jain and his colleagues wondered whether obesity contributes to resistance to anti-angiogenic therapy, which targets the blood supply of tumors. To find out, they analyzed data from a clinical trial of 99 breast cancer patients who were initially treated with the anti-angiogenic drug bevacizumab (an antibody that targets VEGF) and then with chemotherapy. They reported in a paper published in March in *Science Translational Medicine* that responses to anti-VEGF therapy were generally weak, and that obese patients had tumors that were 33% larger compared to individuals who had a BMI below 25. These patients had elevated levels of interleukin 6 (IL-6), which promotes inflammation, and fibroblast growth factor 2 (FGF-2), which also drives angiogenesis—both of which were being produced by fat cells and nearby cells in tumors. They also exhibited the poorest response to anti-VEGF treatment. Mouse models of breast cancer recapitulated these findings. In an ER-β positive model, anti-IL6 treatment restored response to anti-VEGF therapy to levels comparable to that of lean animals; in a triple-negative breast cancer model, targeting FGF-2 had similar effect. The findings could be translated and tested relatively quickly, as inhibitors of both FGF-2 and IL-6 pathways are already available.

EARLY WARNING I

Ovarian and endometrial cancers are typically detected only after they're well advanced. This is partly because there's no quick, easy and reliable method to catch them in their early stages. In a March paper published in *Science Translational Medicine*, Ludwig Johns Hopkins' Nick Papadopoulos and his colleagues report an evaluation of a test, PapSEEK, devised to do just that. Their test relies on fluids collected during routine Pap tests to look for DNA mutations in 18 genes and chromosomal aberrations in cells shed by tumors. Nick and his team studied 1,958 samples obtained from 1,658 women, including 658 endometrial or ovarian cancer patients and 1,002 healthy controls. Pap brush samples were obtained from 382 endometrial cancer and 245 ovarian cancer patients. PapSEEK was nearly 99% specific for cancer. It detected 81% of endometrial cancers (78% early-stage) and 33% of ovarian cancers (34% early-stage). The sensitivity of the test was improved by using a brush that extends further into the cervical canal: rising to 93% for endometrial cancer and 45% for ovarian cancer. When tests using both plasma (liquid biopsy) and Pap brush samples were combined, ovarian cancer detection rose to 63%.

Nick Papadopoulos
Ludwig Johns Hopkins

Ken Kinzler
Ludwig Johns Hopkins

Nick Papadopoulos
Ludwig Johns Hopkins

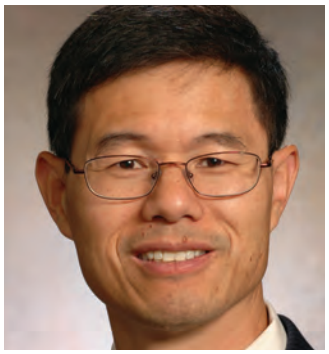
Bert Vogelstein
Ludwig Johns Hopkins

EARLY WARNING II

Researchers led in part by Ludwig Johns Hopkins Co-director Bert Vogelstein and including Co-director Ken Kinzler and Nick Papadopoulos and colleagues at Johns Hopkins evaluated a liquid biopsy for detection of bladder and upper tract urothelial cancer (UTUC). Named UroSEEK, the test looks for chromosomal aberrations and mutations in 11 cancer-associated genes. An early detection cohort supplied urine samples prior to any surgical procedures. A second cohort comprised Taiwanese patients with UTUC who supplied a urine sample prior to surgery for their cancer. A third cohort recruited patients at high risk for recurrence of bladder cancer. The researchers studied 570 patients in the early detection cohort and found UroSEEK detected 83% of patients who developed cancer. When combined with an examination of cells—a currently standard noninvasive test—it detected 95% of such patients. UroSEEK also detected bladder cancer in 71% of patients who showed signs of recurrence; cytology only found 25% of these patients. In the cohort of patients with UTUC, 75% tested positive by UroSEEK, while cytology only detected 10%. The study was published in *eLife* in March and was also led by Kathleen Dickman of Stonybrook University and George J. Netto, now at the University of Alabama.

FLOWER POWER

A team led by Ludwig Chicago's Wenbin Lin and Ralph Weichselbaum has developed a treatment strategy that combines nanoscale metal–organic framework (nMOF)-enabled radiotherapy–radiodynamic therapy (say that five times, quickly!) with checkpoint blockade immunotherapy. The former involves injecting into a single tumor a nanoparticle that schematically resembles a cage of flowers. The rather pretty if prosaically named nMOF, which absorbs radiation much better than does tissue, is also chemically modified to amplify its effect—which is to generate a storm of highly reactive oxygen species that are lethal to cells. But wait, there's more. Wenbin, Ralph and colleagues filled their particular nMOF with an IDO inhibitor, which can disable a common enzymatic defense deployed by tumors against T cells. The researchers report that injecting a single tumor with the nanomaterial and zapping the tumor with low dose radiotherapy resulted in the complete elimination of various types of tumors (including untreated ones) in mice. The research was published in March in *Nature Biomedical Engineering*. Their nanoflower is currently trying its luck against human tumors in a phase 1 clinical trial.



◀ Wenbin Lin
Ludwig Chicago

◀ Ralph Weichselbaum
Ludwig Chicago

Tyler Jacks ▶
Ludwig MIT

NO KRAS ADDICTION

Pancreatic ductal adenocarcinomas (PDACs), which account for 90% of all pancreatic cancers, are very difficult to treat. Since the *KRAS* gene is mutated in 95% of PDACs, it has long been assumed that its cells are "addicted" to the oncogene. A February *Cancer Research* study led by Ludwig MIT's Tyler Jacks, however, calls that assumption into question. Tyler and his colleagues analyzed mouse PDAC cells using a temporally controllable gene silencing system and found that the majority tolerated both acute and sustained loss of KRAS signaling. The cells were slowed by the inhibition of *KRAS* but, surprisingly, did not die. The cells, it turned out, simply rewired their signaling pathways, adapting in nongenetic and reversible ways that altered their morphology, proliferative behavior and ability to seed new tumors. These alterations made them more dependent on being anchored down for survival. But survive they did. So while the study may be sobering to folks developing KRAS inhibitors, its findings open the door to developing combination therapies to undo PDAC's resistance to such therapies.

EPIGENETIC EXPLANATION

Enhancer sequences in the genome do what their name suggests—they enhance the expression of their target genes, which can be very far away on the linear DNA strand. When activated, enhancers sport a standard chemical modification to a component of their chromatin (a term for DNA and its protein packaging) called histone. This modification is known as H3K4me1, and how precisely it works to rev up gene expression has long been unclear. To find out, Ludwig San Diego's Bing Ren and his colleagues conducted experiments with individual structural units of chromatin (called nucleosomes) to explore how H3K4me1 exerts its influence. Their results, published in January in *Nature Genetics*, suggest that it promotes the binding to the enhancer of a protein machine called the BAF complex that remodels chromatin to enable gene expression. They show how this binding occurs through x-ray crystallography and demonstrate that H3K4me1 marks improve the remodeling of nucleosomes. Both the enzymes that deposit the epigenetic mark and the BAF complex it influences have been shown to be tumor suppressors in a variety of cancers.

Bing Ren
Ludwig San Diego

Alexandre Harari
Ludwig Lausanne

George Coukos
Ludwig Lausanne

TUMOR TIL-LING

Ovarian tumors, whose cells are not very extensively mutated, have long proved resistant to immunotherapies. But in a March paper in *Nature Communications*, a team led by Ludwig Lausanne investigator Alexandre Harari and Branch Director George Coukos shows that these tumors still have within them T cells that target novel and often patient-specific mutations to proteins known as neoantigens. They also describe how such T cells can be extracted and selectively grown for use in personalized, cell-based immunotherapies. In their method, the researchers focused on killer T cells that have slipped into the tumor to target cancer cells, referred to as tumor infiltrating T lymphocytes (TILs). They isolated and grew these TILs in a manner that selectively boosted those that reacted vigorously against neoantigens. Comparing T cells from the blood with TILs targeting the same neoantigens, they showed that the TILs were far more functional and lethal to cancer cells. Notably, the researchers found that, using their methods, highly reactive TILs could be obtained from some 90% of the ovarian cancer patients whose tumor samples they examined. This has implications for a broad range of other tumors that, like ovarian cancers, have a low burden of mutation and have similarly resisted immunotherapies.

BEAR TRUTH

A technique known as single-cell RNA sequencing (scRNAseq), which profiles global gene expression in single cells, has revolutionized our ability to detect fine differences between individual cells in a chunk of tissue—a capability essential to every branch of animal and human biology. But given the minute quantities involved in such analyses, the smallest technical errors—both random (say, a gene transcript being missed) and methodological (like errors in sample processing)—can be hugely amplified in the results. And since the single cells assessed can't be retested to confirm initial results (they have been destroyed), it's very hard to account for technical variability in RNAseq data—something that can compromise the integrity of results. To address these issues, Ludwig Oxford's Benjamin Schuster-Böckler and Xin Lu and their colleagues developed a tool named BEARscc. Reported in *Nature Communications* in February, it makes innovative use of controls commonly added to scRNAseq experiments. Based on "spike-ins", trace amounts of RNA of known concentration, BEARscc generates a computational model of the expected technical noise in the data so that it can be accounted for in subsequent analyses. BEARscc is shown to improve the classification of cell types in tissue and aid the sound interpretation of RNAseq data.

Xin Lu
Ludwig Oxford

Benjamin Schuster-Böckler
Ludwig Oxford

A SUFFICIENT INSUFFICIENCY

Ludwig Oxford Director Xin Lu and her team have previously shown that the versatile and ubiquitously expressed iASPP protein, an inhibitor of p63 and the tumor suppressor protein p53, helps regulate not only gene expression but the integrity of the junctions between connected cells as well. They've also shown that mutations of iASPP cause cardiocutaneous syndrome—a cluster of rare genetic disorders characterized by cardiac dysfunction and impaired wound healing. What wasn't clear was whether these changes were due to iASPP's dysfunction in heart and skin cells or due to secondary effects caused by its deficiency in other cell types, like immune cells. In a January paper in *Cell Death and Differentiation*, Xin's team showed by selectively deleting iASPP in heart and skin cells that iASPP dysfunction in these cells themselves is sufficient to induce the characteristic disorders of cardiocutaneous syndrome. The wound healing defects are caused by poor connections between cells and impaired cell migration brought on by iASPP dysfunction. The findings suggest iASPP problems may contribute to skin and heart diseases caused by multiple gene defects as well.

Alex Toker
Ludwig Harvard

EXPLOITING AN ADDICTION

Some breast cancer cells are highly dependent on an external supply of the amino acid methionine. In a December paper in *Science Signaling*, a team led by Ludwig Harvard's Alex Toker sheds light on one mechanism behind that dependency. Alex and his team screened 13 breast cancer cell lines that are addicted to methionine and found that they all had mutations in PIK3CA pathway genes. Turns out that the oncogenic PIK3CA inhibits the activity of another protein, xCT, which imports a molecule involved in the cell's production of a related amino acid named cysteine. When that molecule is in short supply, the cells compensate by pushing another metabolic intermediate—homocysteine—toward cysteine production. Trouble is, homocysteine is also used to make methionine, so the cells are forced to import that amino acid to survive. The researchers showed that exposing breast cancer cells encoding a normal PIK3CA to a drug (sulfasalazine) that inhibits xCT made those cells dependent on methionine import as well. The new findings raise the possibility of treating tumors by triggering methionine dependency using sulfasalazine or other drugs that hit xCT. Alex and his team are exploring that possibility.

Ralph Weichselbaum
Ludwig Chicago

RADIATION'S STING

Some 40% of large tumors become resistant to radiotherapy. In a November paper in *Nature Communications*, a team led by Ludwig Chicago Co-director Ralph Weichselbaum and Yang-Xin Fu of UT Southwestern Medical Center describes a molecular mechanism of such resistance that involves crosstalk between cancer cells and suppressors of immune responses known as monocytic myeloid-derived suppressor cells (M-MDSCs). The mechanism involves a molecule in cells named STING, which detects DNA fragments generated by radiation (and, ordinarily, viral infection). STING drives the production of immune factors known as type 1 interferons (IFNs). At first, these factors boost the activation of killer T cells, which attack cancerous cells. But over time, STING also activates the production of a protein that binds a receptor named CCR2 on M-MDSCs. This draws the suppressive cells into the tumor, where they quell the T cell attack. The researchers showed that treating normal tumor-bearing mice with a STING-activating drug and anti-CCR2 antibodies almost eliminated resistance to radiotherapy. Drug companies are already developing cancer drugs that activate STING and others that block CCR2, so the finding has significant translational potential.

Lana Kandalaft
Ludwig Lausanne

George Coukos
Ludwig Lausanne

Alexandre Harari
Ludwig Lausanne

TOWARD TAILOR-MADE TREATMENTS

A study led by Ludwig Lausanne's Lana Kandalaft, George Coukos and Alexandre Harari reveals that an entirely novel and personalized cancer vaccine induces clinically effective immune responses in patients receiving a combination of standard therapies for recurrent, advanced ovarian cancer—which has so far proved utterly resistant to immunotherapy. The personalized vaccine was made by digesting tumor samples from each patient, treating the resulting slurry with acid and feeding it to each patient's own dendritic cells, which "show" T cells the antigens that should guide their attack. These activated dendritic cells were then injected directly into the lymph nodes of each corresponding patient. The researchers reported in an April issue of *Science Translational Medicine* that eight of the ten women who received the vaccine along with cyclophosphamide and bevacizumab—routine treatments for recurrent ovarian cancer that also happen to weaken the tumor's immune defenses in distinct ways—were still alive after two years. By comparison, fewer than half of a group of 56 patients who received standard treatment at the clinic were alive at the two-year mark. Immunologic analyses revealed that T cells elicited by the vaccine not only recognized a broad spectrum of neoantigens (which are unique to each patient) but were far more sensitive to lower levels of those antigens, and more fiercely activated by them.

Charlotte Ariyan
Ludwig MSK

Jedd Wolchok
Ludwig MSK

Alexander Rudensky
Ludwig MSK

ONE-TWO PUNCH

A study led by Ludwig MSK's Charlotte Ariyan, Jedd Wolchok and Alexander Rudensky found that localized, high-dose chemotherapy followed by systemic treatment with the checkpoint blockade immunotherapy ipilimumab can induce significant and durable anti-cancer responses in patients with relatively advanced melanoma. Published in a January issue of *Cancer Immunology Research*, the study enrolled 26 patients whose cancers were "in transit"—tumors spreading through a limb toward the rest of the body—or, in a few cases, had already become systemic (stage IIIB/IIIC, and stage IV, respectively). The researchers employed a surgical procedure in which the cancerous limbs of patients are isolated with a tourniquet and infused with high doses of chemotherapy. They then followed up with an average of three systemic doses of ipilimumab. Some 85% of patients receiving the combination treatment had significant anti-tumor responses within 3 months, with 62% having no detectable tumors at that point and 23% showing significant regressions. Overall, the cancer had not progressed any further in 58% of the patients a year later, and median progression-free survival had not been reached 36 months after treatment.

JOHANNA JOYCE
LUDWIG LAUSANNE

What are the biggest questions you aim to answer in your lab?
How do cancer cells establish a dialogue with normal cells within their microenvironments? How does that conversation change during the course of cancer progression and metastasis? How is that dialogue affected by different therapeutic interventions? We want to answer these questions in different cancers, with a recent emphasis on brain malignancies, both primary gliomas as well as brain metastases that originate from the breast, lung and other organs and disseminate to the brain. Brain metastases are very challenging targets for therapy and prevention. By developing a comprehensive understanding of the complex and interconnected microenvironmental landscape of brain malignancies, we're getting closer to understanding this very intricate communication involving many different cell types within the brain microenvironment. We can use these datasets to then identify weak points and therapeutically intervene, and either stop that communication altogether or change it such that the normal cells are re-educated to actively fight the cancer.

Did you have any early influences that put you on the path to a career in science?
My teachers and professors in high school and university were instrumental in reinforcing my innate interest in science. I remember one high school teacher in

> One of the fun parts of doing science is in fact when the outcome is not what you expect it to be. The obstacles or roadblocks force you to think about a problem more creatively.

particular—Mr. Bennett—who had an infectious enthusiasm for chemistry, which he was able to convey to his students. His classes were key in reinforcing my own enthusiasm for science. As an undergraduate at Trinity College in Dublin, we had outstanding professors in genetics. They taught us to think about and approach fundamental questions of biology from a genetic perspective and were very adept at unlocking many of the mysteries of biology and the beauty of genetics.

When you were 14, you moved from London to the Irish countryside. Was the transition difficult?
At the beginning, yes it was quite an adjustment. At 14, I was starting to become independent and I wasn't happy to leave my friends behind. Now when I look back though and see how my life has worked out, I'm really pleased that my family made that decision, as it ultimately benefited all of us.

Did it influence your decision to pursue science?
The Irish school system allowed me to explore a wider range of subjects—history, geography, French and English in addition to the sciences—than I would have been able to in the more restricted English school system. In England, I would have had to concentrate just on the four sciences. In my opinion, 14 is far too young to specialize because at that age, you tend to change your mind pretty frequently about what you want to be when you grow up. But I'm fortunate in that I've always been drawn to science. It's what I always found most fascinating at school, and having many different perspectives when thinking about scientific problems can be influenced by input from other subjects—so that was definitely another good outcome of our move to Ireland.

How would you describe yourself as a child? Introspective or outgoing, bookish or athletic, intense or laid-back?
Probably none of those strict 'either or' adjectives actually apply. I would say that I was very curious as a child, and an avid reader. I devoured books. I was always asking a lot of questions and always wanted to discover new things and walk into the unknown.

You've been quoted as saying that you tend to approach life as a game of Snakes and Ladders. Does science ever feel like the board game?
Very much. Often, you feel as if you take two steps forward and one step back. You're advancing on a project and all of a sudden you uncover something that forces you to take a step back and reflect on what the data are telling you. But I think of science more as a journey where we encounter obstacles along the way. They can be frustrating but I see them as challenges. One of the fun parts of doing

science is in fact when the outcome is not what you expect it to be. The obstacles or roadblocks force you to think about a problem more creatively and approach it from different angles.

What has been your best or most satisfying moment in the lab?
About seven years ago, several lab members were collaborating on a project targeting macrophages in an animal model of gliomas (brain tumors). Glioblastoma is a very challenging disease to treat and they found that using an inhibitor of macrophages was essentially curing the mice. These were animals that were days away from having to be sacrificed because their tumor was growing rapidly in the brain. Yet the treatment with a specific drug completely reversed the process. The tumors regressed and the mice survived. That effect was so striking, so profound, especially when you saw the effect that it had on the animals' behavior. That was a very special moment because we not only saw its potential implications but also, in retrospect, because that particular finding set my lab on the path to where we are now, which is working on trying to understand the brain tumor microenvironment as one of our major challenges. Many people in my lab were involved in different ways on that project and we certainly benefited from working on it together as a team. Of course, everybody hopes to have one of those moments in their scientific career and I've been fortunate in having several of those, including in my research as a student and as a postdoc. But that is one that will always stay with me.

Who are the scientists, living or dead that you admire? If you could, whom would you work with?
Barbara McClintock. She was a geneticist who won the Nobel Prize in 1983 for discovering transposable elements—the ability of special DNA sequences to move around in the genome. She was inspirational in a number of ways. She made multiple landmark discoveries, some of which took decades to be recognized and appreciated. She was ignored by her peers and for years they dismissed her findings. But she never gave up and continued to work on her own. Here was a scientist who was doing creative, transformational research, being ignored, being told that she was wrong and yet she was never discouraged. She just kept going. Her research has had a fundamental influence on much of what we understand about the mechanisms underlying inheritance and her discoveries have had an effect on everything from genetic engineering to cancer research. She was also a scientist

at a time when it wasn't easy for a woman to pursue a career in science and yet she was so single-minded and persistent, she prevailed. She was a true innovator in her field and is an inspiration to aspiring scientists.

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Working in science is wonderful and challenging but is not without drawbacks. What has been a particular challenge to you?
As you progress in your career, you have many more demands on your time—you have responsibilities to the people in your lab, you have administrative responsibilities, you have to travel a lot. And this is in addition to having a family. These are challenges every professional faces. When I say yes to something I only say yes if I'm able to do it well. And for me that means more and more having to say no. I have to ask myself where can I have the most impact, so I'm very selective about which committees I'll sit on, which conferences I'll attend or organize. You have to be very organized and I basically schedule down to the hour. When I'm at work I try to be as productive as possible. When you have young children, you have to leave at a certain time and that is a great external force to then be incredibly efficient with your time while at work. This is of course true for men as well as women.

Have you ever followed up on a failure?
In my lab, we aim to explore big questions and test open-ended hypotheses. That way, even if the answer ultimately is negative, it's still as informative as if we had gotten a positive outcome. You don't end up with failures as such because you asked a question that was worth answering to begin with. I'd almost characterize a 'failure' as a new challenge resulting from an unexpected finding that then needs to be explored further.

There has been a lot of discussion in the media lately about women in science. What has been your experience as a female scientist?
It's complicated. I don't consider myself to be a 'woman scientist' but a scientist who happens to be a woman. We never talk about male scientists. Never. But we talk about women scientists all the time. Bottom line, there are not enough senior scientists who are women. Young female postdocs don't see enough women in the career stages ahead of them, and they are then led to believe it's too challenging and not compatible with having a family or interests outside of the lab. We need to encourage more female postdocs to apply for faculty positions. This is precisely where the drop off is happening. It's not as if there are women applying and they are not getting the positions at the same rate as men. It's just that not as many women are applying. The University of Lausanne sees this is an issue and has set a target of having women fill 40% of new faculty posts by 2020. The universities in Switzerland and the Swiss National Science Foundation are also implementing various programs to try and change these metrics and I think they should be commended for this. Hopefully these programs will provide constructive and informative examples for initiatives that can be considered and implemented across the world.

How do you see your role in helping to mentor young scientists?
Mentoring is very important at the postdoc level and in particular during the transition to independence. Everyone needs a supportive community of people who are pleased to write letters when you're applying for grants, nominate you for awards and suggest you as a speaker at conferences. I remember how it was for me at that early stage in my career and the wonderful support that I received from a number of senior colleagues, and I'm a firm believer that it's really important to give back when you're in a position to do so.

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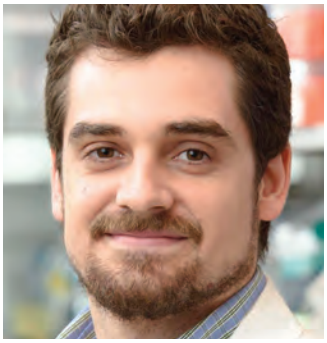
You are also a professor at the University of Lausanne. Can you tell us a little bit about your teaching philosophy and what brings you the most joy in interacting with students?
My teaching philosophy is to engage my students—not lecture to them. My classes are very interactive and foster a lot of discussion. I think that's more enjoyable and more informative for them and also far more rewarding for me. I don't want them to just absorb a particular topic but to critically assess what they are learning, which has far more of an impact on their understanding and retention than just the traditional, rote learning approach.

How do your teaching and research experiences intersect?
Quite a bit of the teaching that I do focuses on topics that we basically research in my lab—the processes of cancer invasion and metastasis, and the critical importance of the tumor microenvironment. When I teach, I try to use language that's accessible, simple and straightforward. I aim to do away with the jargon and distill the concepts down to the basic principles. This forces me to then reflect on some of the research that we're doing in the lab and what's happening in the field. Too often we get caught up in dogma and in the preexisting literature. When I'm teaching I can take a step back and reframe the way I think about questions in the context of how I explain concepts and theories to my students. In that way, teaching and research can actually be quite closely intertwined and definitely feed off each other.

What else do you love in life?
I love spending time with my husband and kids—outside of our lives in science, much of our time is spent outdoors, especially around Lake Geneva. Switzerland is a beautiful place to raise children, and we feel very privileged to live here.

How can scientists best reach out with social media?



Social media sites represent a great platform for scientists to engage with the general public. Through articles or blog posts written in an intelligible and colloquial manner, scientists can inform the public about relevant scientific discoveries and bring awareness to the potential transformative impact of science on society. In the era of "alternative facts", dissemination of evidence-based argumentation is critical and should be strongly supported.

LUIS FELIPE CAMPESATO
Ludwig MSK

Social media lets us climb down from our ivory tower so that our voices can be heard. It's a direct outlet that lets us discuss our work with the world. Cancer research is our passion and social media lets us communicate why we're so excited about our work.

SEAN FANNING
Ludwig Chicago

Whether it is promoting our work, looking for new career and funding opportunities or hiring people, social media is increasingly becoming our choice for global outreach. We need to build effective networks and, at the same time, help our peers join these virtual communities. In my opinion, the key to our success is in being open to new possibilities.

ANITA ROY
Ludwig Brussels

Many scientists, myself included, could do a better job of reaching out on social media. Promoting and highlighting findings from sound scientific studies may well help to counter the abundance of half-truths and "scientific" propaganda that flood the Internet. But I would caution that simply re-tweeting or posting is not enough, and that we must remain personally engaged in our local communities.

ARTHUR W. LAMBERT
Ludwig MIT

Required reading

Ludwig Chicago

Molecular Cancer Therapeutics 2018 April 17
JAK2 inhibitor SAR302503 abrogates PD-L1 expression and targets therapy resistant non-small cell lung cancers.

Pitroda S, Stack M, Liu GF, Song SS, Chen L, Liang H, Parekh AD, Huang X, Roach PB, Posner MC, Weichselbaum RR, Khodarev NN.

Nature Biomedical Engineering 2018 March 26
Low-dose X-ray radiotherapy–radiodynamic therapy via nanoscale metal–organic frameworks enhances checkpoint blockade immunotherapy

Lu K, He C, Guo N, Chan C, Ni K, Lan G, Tang H, Pelizzari C, Fu Y-X, Spiotto MT, Weichselbaum RR, Lin W.

Nature Communications 2017 November 23
Host STING-dependent MDSC mobilization drives extrinsic radiation resistance.

Liang H, Deng L, Hou Y, Meng X, Huang X, Rao E, Zheng W, Mauceri H, Mack M, Xu M, Fu YX, Weichselbaum RR.

Ludwig Harvard

Cell 2018 April 5
Profound tissue specificity in proliferation control underlies cancer drivers and aneuploidy patterns.

Sack LM, Davoli T, Li MZ, Li Y, Xu Q, Naxerova K, Wooten EC, Bernardi RJ, Martin TD, Chen T, Leng Y, Liang AC, Scorsone KA, Westbrook TF, Wong KK, Elledge SJ.

Science Translational Medicine 2018 March 14
Obesity promotes resistance to anti-VEGF therapy in breast cancer by up-regulating IL-6 and potentially FGF-2.

Incio J, Ligibel JA, McManus DT, Suboj P, Jung K, Kawaguchi K, Pinter M, Babykutty S, Chin SM, Vardam TD, Huang Y, Rahbari NN, Roberge S, Wang D, Gomes-Santos IL, Puchner SB, Schlett CL, Hoffmman U, Ancukiewicz M, Tolaney SM, Krop IE, Duda DG, Boucher Y, Fukumura D, Jain RK.

Science Signaling 2017 Dec 19
Oncogenic PI3K promotes methionine dependency in breast cancer cells through the cystine-glutamate antiporter xCT.

Lien EC, Ghisolfi L, Geck RC, Asara JM, Toker A.

Ludwig Johns Hopkins

Science Translational Medicine 2018 March 21
Evaluation of liquid from the Papanicolaou test and other liquid biopsies for the detection of endometrial and ovarian cancers.

Wang Y, Li L, Douville C, Cohen JD, Yen TT, Kinde I, Sundfelt K, Kjær SK, Hruban RH, Shih IM, Wang TL, Kurman RJ, Springer S, Ptak J, Popoli M, Schaefer J, Silliman N, Dobbyn L, Tanner EJ, Angarita A , Lycke M, Jochumsen K, Afsari B, Danilova L, Levine DA, Jardon K, Zeng X, Arseneau J, Fu L, Diaz LA Jr, Karchin R, Tomasetti C, Kinzler KW, Vogelstein B, Fader AN, Gilbert L, Papadopoulos N.

Elife 2018 March 20
Non-invasive detection of urothelial cancer through the analysis of driver gene mutations and aneuploidy.

Springer SU, Chen CH, Rodriguez Pena MDC, Li L, Douville C, Wang Y, Cohen JD, Taheri D, Silliman N, Schaefer J, Ptak J, Dobbyn L, Papoli M, Kinde I, Afsari B, Tregnago AC, Bezerra SM, VandenBussche C, Fujita K, Ertoy D, Cunha IW, Yu L, Bivalacqua TJ, Grollman AP, Diaz LA, Karchin R, Danilova L, Huang CY, Shun CT, Turesky RJ, Yun BH, Rosenquist TA, Pu YS, Hruban RH, Tomasetti C, Papadopoulos N, Kinzler KW, Vogelstein B, Dickman KG, Netto GJ.

Ludwig Lausanne

Science Translational Medicine 2018 April 11
Personalized cancer vaccine effectively mobilizes antitumor T cell immunity in ovarian cancer.

Tanyi JL, Bobisse S, Ophir E, Tuyaerts S, Roberti A, Genolet R, Baumgartner P, Stevenson BJ, Iseli C, Dangaj D, Czerniecki B, Semilietof A, Racle J, Michel A, Xenarios I, Chiang C, Monos DS, Torigian DA, Nisenbaum HL, Michielin O, June CH, Levine BL, Powell DJ Jr., Gfeller D, Mick R, Dafni U, Zoete V, Harari A, Coukos G, Kandalaft LE.

Nature Communications 2018 March 15
Sensitive and frequent identification of high avidity neo-epitope specific CD8 + T cells in immunotherapy-naive ovarian cancer.

Bobisse S, Genolet R, Roberti A, Tanyi JL, Racle J, Stevenson BJ, Iseli C, Michel A, Le Bitoux MA, Guillaume P, Schmidt J, Bianchi V, Dangaj D, Fenwick C, Derré L, Xenarios I, Michielin O, Romero P, Monos DS, Zoete V, Gfeller D, Kandalaft LE, Coukos G, Harari A.

Ludwig MIT

Science Translational Medicine 2018 Apr 11
The systemic response to surgery triggers the outgrowth of distant immune-controlled tumors in mouse models of dormancy.

Krall JA, Reinhardt F, Mercury OA, Pattabiraman DR, Brooks MW, Dougan M, Lambert AW, Bierie B, Ploegh HL, Dougan SK, Weinberg RA.

Cancer Research 2018 February 15
Adaptive and reversible resistance to Kras inhibition in pancreatic cancer cells.

Chen PY, Muzumdar MD, Dorans KJ, Robbins R, Bhutkar A, Del Rosario A, Mertins P, Qiao J, Schafer AC, Gertler F, Carr S, Jacks T.

Ludwig MSK

Journal of Clinical Investigation 2018 April 2
PD-L1 in tumor microenvironment mediates resistance to oncolytic immunotherapy.

Zamarin D, Ricca JM, Sadekova S, Oseledchyk A, Yu Y, Blumenschein WM, Wong J, Gigoux M, Merghoub T, Wolchok JD.

Cancer Immunology Research 2018 February
Robust antitumor responses result from local chemotherapy and CTLA-4 blockade.

Ariyan CE, Brady MS, Siegelbaum RH, Hu J, Bello DM, Rand J, Fisher C, Lefkowitz RA, Panageas KS, Pulitzer M, Vignali M, Emerson R, Tipton C, Robins H, Merghoub T, Yuan J, Jungbluth A, Blando J, Sharma P, Rudensky AY, Wolchok JD, Allison JP.

Required reading

Ludwig Oxford

Nature Communications 2018 March 22

BEARscc determines robustness of single-cell clusters using simulated technical replicates.

Severson, DT, Owen RP, White MJ, Lu X, Schuster-Böckler B.

Cell Death and Differentiation 2018 January 19

Cell autonomous role of iASPP deficiency in causing cardiocutaneous disorders.

Dedeić Z, Sutendra G, Hu Y, Chung K, Slee EA, White MJ, Zhou FY, Goldin RD, Ferguson DJP, McAndrew D, Schneider JE, Lu X.

Ludwig San Diego

Nature Genetics 2018 January

Identification of H3K4me1-associated proteins at mammalian enhancers.

Local A, Huang H, Albuquerque CP, Singh N, Lee AY, Wang W, Wang C, Hsia JE, Shiau AK, Ge K, Corbett KD, Wang D, Zhou H, Ren B.

Ludwig Stanford

Nature Medicine 2018 May

Potent antitumor efficacy of anti-GD2 CAR T cells in H3-K27M+ diffuse midline gliomas.

Mount CW, Majzner RG, Sundaresh S, Arnold EP, Kadapakkam M, Haile S, Labanieh L, Hulleman E, Woo PJ, Rietberg SP, Vogel H, Monje M, Mackall CL.

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