

LUDWIG LINK

SEPTEMBER 2017

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A conversation with Ludwig's deputy scientific director

LUDWIG CANCER RESEARCH

LIFE-CHANGING SCIENCE

LUDWIG LINK ludwigcancerresearch.org **SEPTEMBER 2017**

LETTER



We've put out quite a few Ludwig Link issues over the past few years and the one thing I've never had to worry about is finding sufficient material for the newsletter.

Our research briefs in this issue are as intriguing as ever. Papers

published by your colleagues in the past few months include reports of an elegant nanotechnology to detect tiny ovarian tumors and a paradigm-shifting discovery about the role played by circular, extrachromosomal fragments of DNA in tumor evolution.

You'll also find in these pages the usual collection of honors bestowed on Ludwig scientists, including richly deserved recognitions for pioneering contributions to immunotherapy and for general excellence in research. Our Q&A this time around is with Ludwig's Deputy Scientific Director, Bob Strausberg, who tells us about his career, his aspirations for Ludwig's research program and his fascinations. (Spoiler alert: Woodstock is mentioned.)

We also include in this issue an update on one of Bob's favorite projects: the Cancer Prevention Initiative we've launched in partnership with the Conrad N. Hilton Foundation. It has now expanded to include three research teams at Ludwig Oxford.

Since this is the 15th anniversary of the mapping of the human genome, we asked a few of our scientists to tell us about what they think is the biggest challenge in analyzing the cancer genome. Their answers are on page 22.

Hope you enjoy this issue of Ludwig Link.

Sincerely,

Rachel Steinhardt Vice President for Communications

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On the cover: Ping-Chih Ho and Wan-Chen Cheng, Ludwig Lausanne

Awards and distinctions



Thierry Boon Ludwig Brussels

FOR FOUNDATIONAL DISCOVERIES

Thierry Boon of Ludwig Brussels received the Lifetime Achievement Award at the 4th Immunotherapy of Cancer Conference (ITOC) in Prague, in March. The conference is organized jointly by the Society for Immunotherapy of Cancer and the Cancer Drug Development Forum. An early proponent of immunotherapy, Thierry made fundamental discoveries that helped lay the foundations of the field. In 1982, he and Aline van Pel made a landmark discovery that specific immunity to spontaneous tumors could be induced by vaccinating mice with tumor cells. This showed that spontaneous tumors were not inherently deficient in

tumor antigens, just that they failed to stimulate an effective immune response. That failure could, then, be overcome by vaccination—a hypothesis that continues to be evaluated in numerous preclinical and clinical studies. In 1991, Thierry—who directed Ludwig Brussels from 1978 to 2009—and his colleagues at the Branch, Benoît Van den Eynde and Pierre van der Bruggen, identified the first human tumorspecific antigen recognized by T cells. In a statement announcing the award, the ITOC called Thierry, "one of the most renowned researchers in the field of anticancer vaccines."

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FOR ILLUMINATING A MICROCOSM

Johanna Joyce of Ludwig Lausanne has been elected to EMBO, the prestigious European organization of researchers that promotes excellence in the life sciences on the continent. Johanna's election recognizes her significant contributions to our understanding of how immune and other noncancerous cells support the survival, progression and metastasis of tumors. Her groundbreaking discoveries on how brain tumors recruit macrophages to their environment and transform the immune cells into unwitting allies have broad implications for cancer research and therapy. Her lab continues to probe the various contributions of the tumor microenvironment and its molecular components to therapeutic resistance.

Johanna is also part of an international team of researchers led by Greg Hannon of Cambridge University that has won a £20 million Grand Challenge award from Cancer Research UK. The team's task in the five-year term of the grant is to develop an interactive virtual reality map of breast cancer that identifies and deeply analyzes every cell in such tumors. The researchers-ranging from computer scientists to engineers to immunologistsaim to construct a 3D model of a breast tumor that can be studied in virtual reality, allowing researchers to walk into the tumor and find out all they want about each of its cells and their roles and interactions with others.



Johanna Joyce Ludwig Lausanne

Awards and distinctions



Teamwork wins! Ludwig scientists from Johns Hopkins and Melbourne at the Opening Ceremony of the AACR Annual Meeting in Washington, DC, in April.

FOR TEAMWORK

Ludwig scientists from John Hopkins and Ludwig Melbourne alumni were awarded the prestigious American Association for Cancer Research (AACR) Team Science Award for their collective efforts in developing liquid biopsy tests for the detection, diagnosis, prevention and treatment of cancer. The team members include Bert Vogelstein, Luis Diaz, Nishant Agrawal, Chetan Bettegowda, Frank Diehl, Peter Gibbs, Stanley Hamilton, Ralph Hruban, Hartmut Juhl, Isaac Kinde, Kenneth Kinzler, Martin Nowak, Nickolas Papadopoulos, David Sidransky, Jeanne Tie and Victor Velculescu. The award was established by the AACR and Eli Lilly and Company to acknowledge the growing importance of interdisciplinary teams to the understanding of cancer and the translation of research discoveries into clinical applications. The award, along with a \$50,000 honorarium, was presented in April during the Opening Ceremony of the AACR Annual Meeting 2017 in Washington D.C.

Awards and distinctions



Sangeeta Bhatia Ludwig MIT



Junying Yuan Ludwig Harvard

FOR GENERAL EXCELLENCE

Ludwig MIT's Sangeeta Bhatia and Ludwig Harvard's Junying Yuan were elected to the National Academy of Sciences in recognition of their "distinguished and continuing achievements in original research." A physician and bioengineer, Sangeeta led the development of human microlivers that are widely deployed today to study tissue regeneration and examine the effects of drugs and infectious agents on the human liver. She has also has contributed significantly to the application of nanotechnology and synthetic biology for cancer diagnostics and drug delivery. Junying is a pioneer in the field of cell death. Her works provided pivotal insights into the fundamental mechanisms that regulate cell death in mammalian cells, including both apoptosis and necroptosis, and their relevance in major human inflammatory and neurodegenerative disorders and cancers. She is credited with the discovery of the role of caspases in mediating apoptosis as well as a programmed form of necrotic cell death known as necroptosis. She was instrumental in identifying a key instigator of nerve cell damage in people with amyotrophic lateral sclerosis, or ALS, a progressive and incurable neurodegenerative disorder.

News roundup

MISSILE DEFENSE

A team led by Stanford computer scientists David Dill and Subarna Sinha, who has moved to SRI International, Ludwig Stanford's Ravi Majeti and his fellow Daniel Thomas crafted an algorithm named MiSL (pronounced "missile") to identify pairs of genes that, when simultaneously inactivated, kill cancer cells. Such gene pairs, known as synthetic lethals, can be exploited for therapy: If one is mutated in cancer cells, hitting the protein product of the other with a drug would kill the cancer cell while sparing healthy cells. In their study, published in May in Nature Communications, the researchers applied MiSL to analyze more than 3,000 mutations in a dozen types of tumors. MiSL found 145,000 potentially synthetic lethal partner genes, many of which could be exploited for therapy. The researchers identified 89 potential synthetic lethal partners for an IDH1 mutation associated with acute myeloid leukemia and several other cancers, 17 of which they suspect could be targeted by drugs that are already available or under development. They also showed in lab experiments that exploiting one of those partnerships-by also disrupting a gene called ACACAwas selectively deadly to leukemia cells.





Ravi Majeti Ludwig Stanford

Daniel Thomas Ludwig Stanford

TUNED TO THRIVE

Cancer cells have to reengineer their metabolism quite extensively to obtain the raw materials and energy sources they require to proliferate and survive in the tough environs of a tumor. In a paper published in July in Molecular Cell, a team led by Ludwig San Diego's Paul Mischel identifies a new mechanism by which cancer cells "tune" their uptake and utilization of amino acids-the building blocks of proteins-in response to growth factor and environmental cues. The mechanism involves mTORC2, a core component of a signaling pathway that drives the growth of brain, lung and breast cancers. They report that mTORC2 adds a phosphate to a specific site on a protein

called xCT to control its activity. xCT transports specific amino acids across the cell membrane and is known to play a key role in cancer. Paul and his team show that this specific interaction between mTORC2 and xCT is a switch that triggers a cascade that helps tumor cells respond to changes in the environment-allowing them to hoard amino acids to drive tumor growth when resources are abundant, or use them as a cushion against stress in tougher times. The study provides critical insight into how growth signals in cancer cells are linked to their metabolic adaptations, an understanding important to the development of new types of cancer therapy.



Paul Mischel Ludwig San Diego





Irv Weissman Ludwig Stanford

A CHECKPOINT TWOFER

Many tumor cells express relatively large numbers of a defensive protein known as PD-L1 on their surface. The protein binds another, PD-1, that is expressed on the surface of killer T cells. This interaction promptly snuffs out the T cell attack. Checkpoint blockade antibodies have been approved to target these proteins and fuel a therapeutic killer T cell response against certain tumors. In a May Nature paper, Ludwig Stanford Director Irv Weissman and his colleagues report that the antibodies also work on another soldier of the immune system: the macrophage, a type of white blood cell that, when activated, gobbles up cancer cells. Irv and his team found that tumors induce

the expression of PD-1 in macrophages that enter their precincts, and that PD-1 expression in these macrophages increases as the tumor advances. They also show that anti-PD-1 antibodies restore the macrophage's appetite for cancer cells. It isn't yet clear what share of checkpoint blockade's successes in the clinic might be attributed to macrophages (as compared to T cells), but the finding could lead to improved combination therapies for cancer that activate both types of immune cells. It also suggests that anti-PD-1/PD-L1 therapies may be effective against a wider variety of cancers than previously suspected.

MOTOR MALFUNCTION

Neuroblastoma, the third most common malignancy in children, accounts for almost 15% of childhood cancer fatalities. Yet, paradoxically, it also has the highest rate of spontaneous regression of any human cancer. Researchers already know that high expression of a gene named TRKA, the receptor for nerve growth factor, is associated with favorable outcomes and regression. Meanwhile, low TRKA expression and the loss of the bottom tip on one copy of chromosome 1 (1p36 deletion) are among the phenomena associated with a worse prognosis. Susanne Schlisio and her colleagues at Ludwig Stockholm previously demonstrated that the gene for a protein motor named KIF1B β , located

on chromosome 1p36, is a candidate neuroblastoma tumor suppressor. In a Genes & Development paper published in May, Susanne and her team show that KIF1B β is required for the appropriate formation-or differentiation-of neural precursors because the protein is involved in transporting TRKA to the cell surface during development. The researchers found that KIF1Beta mutations associated with neuroblastoma impair this transport and that loss of the gene in the mouse sympathetic nervous system mirrors its loss in neuroblastoma tumors. They report that the defective differentiation of neural precursor cells is an effect of KIF1B β loss that contributes to poor prognosis.



Susanne Schlisio Ludwig Stockholm

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WHAT'S CIS?

The human genome brims with DNA sequences—several million, in fact—that do not encode proteins but do profoundly influence a cell's biology by enhancing or silencing their expression. Mapping the location of these *cis*-regulatory elements is one thing, figuring out their function, quite another. And doing that on a large scale is not a task for the squeamish. Ludwig San Diego's Bing Ren and his team nonetheless took it on. Their June paper in *Nature Methods* reports a highly scalable method named *Cis* Regulatory Element Scan by Tiling-deletion and sequencing (CREST-seq) that knocks out DNA sequences from the genome to detect and discover the function of *cis*-regulatory sequences. The researchers demonstrated the utility of this method by functionally mapping 45 *cis*-regulatory sequences in a 2 million base stretch of the genome that is a key chromosomal locus in human embryonic stem cells. It will doubtless be put to good use in cancer research, since mutations to *cis*regulatory sequences play an important role in the evolution of malignancies.



Bing Ren Ludwig San Diego



Frank Furnari Ludwig San Diego



Jorge Benitez Ludwig San Diego

PRODUCT OF TWO NEGATIVES

The disruption of a tumor suppressor gene is a common driver of tumor growth. Now, in a Nature Communications paper published in May, a team led by Ludwig San Diego's Frank Furnari has shown how disrupting two of them can, counterintuitively, have the opposite effect. Their study demonstrates that when the tumor suppressor gene PTEN is deleted and no longer able to regulate cell growth in glioblastoma multiforme (GBM) brain tumors, disrupting another tumor suppressor, DAXX, slows the growth of GBM tumors in mice. The DAXX gene produces a protein of the same name whose job is to chaperone a protein called H3.3 to its appropriate place in chromatin (the term for DNA and its protein packaging). H3.3 is often

found near potentially cancer-causing genes, or oncogenes, where it suppresses their expression. The discovery that PTEN interacts with DAXX indicates that it can suppress oncogenes by affecting H3.3 binding to chromatin, and Frank along with Jorge Benitez, the lead author on the publication, found that it indeed increases the deposition of DAXX and H3.3 on chromatin. When PTEN is deleted, not enough H3.3 gets to the right spots on chromatin to block oncogene expression. But if both genes are silenced, H3.3 is free to bind to chromatin and silence its target genes, slowing tumor growth. Frank and his team are interested in seeing whether they can induce the same effect by disrupting the interaction with drugs.

TO TREAT HER BRAIN METASTASES

Although targeted therapies have scored successes across an array of cancers, they have not been as effective in controlling brain metastases. Up to half of all patients treated with targeted therapies for breast cancer driven by overexpression of the HER2 gene eventually develop brain metastases, which for this reason tend to be fatal. In a study published in Science Translational Medicine in May, a research team led in part by Ludwig Harvard's Rakesh Jain identified a novel mechanism of resistance to therapies that target HER2overexpressing cancers with an anti-HER2 antibody trastuzumab (Herceptin) or by hitting a signaling protein it activates called

PI3K. Rakesh and his colleagues show that HER3, a signaling protein overexpressed in brain metastases that participates in the same signaling pathway as HER2, plays a significant role in resistance to both HER2- and PI3K-targeting therapies. The researchers also suggest a treatment strategy to overcome this resistance. While neither a drug that targets HER3 nor one that interferes with the interaction between HER2 and HER3 were able to slow the growth of brain metastases, combined treatment with both an anti-HER2 and an anti-HER-3 drug significantly slowed tumor growth and improved survival.



Rakesh Jain Ludwig Harvard

TO SHARE AND SHARE ALIKE

When a cell divides, it aligns its duplicated chromosomes just so along its equator and uses protein cables known as microtubules to reel a copy of each to its opposite poles. This "spindle apparatus" ensures that each daughter cell gets an identical complement of chromosomes. Cancer cells do this rather sloppily, which is one reason they often harbor odd numbers of chromosomes. Ludwig San Diego's Arshad Desai and his colleagues recently published a pair of papers on how healthy cells orchestrate key steps of this process. The first, published in May in Developmental Cell, examined how the connection between the microtubule and a central element of the relevant chromosomal machinery, known as the kinetochore, is regulated. They show that in the fertilized egg of the roundworm C. elegans, the protein factor that stabilizes links between the kinetochore and microtubules-the Ndc80 complexdoes so via another factor known as the Ska complex, not directly, as the model hitherto held. The recruitment of the Ska complex, which stabilizes the connection by constraining the movement of the chromosomes, is in turn regulated by a family of proteins known as the Aurora kinases.

To ensure their accurate distribution to daughter cells while this goes on,

chromosomes hold the cell in mitosis until they are all properly connected to the spindle apparatus. In the second paper, published in July in Genes & Development, Arshad and his team looked at the dynamics of cell division at this crucial stage. They report that chromosomes can also accelerate progression through mitosis-and that the same molecular interface is employed for both the brake and the accelerator mechanisms. This suggests that the balance between the two effects must be controlled by an external influence. They propose that this external factor is likely the connection to the spindle apparatus: when chromosomes are not connected, their kinetochores generate a "wait" signal by recruiting the key activator (Cdc20) of the enzyme that drives cells out of mitosis (APC/C). They do this by recruiting Cdc20 to catalyze formation of a protein complex that inhibits the APC/C; when the kinetochores connect stably to microtubules, they employ the very same binding site to recruit Cdc20, but now modify it to activate the APC/C instead. This mechanism ensures sufficient time for accuracy in chromosome distribution while minimizing the time spent in mitosis-a phase of cellular life when cells are especially vulnerable, since many vital activities like protein production and secretory functions are put on hold.



Arshad Desai Ludwig San Diego

NICHE MATTERS



Tyler Jacks Ludwig MIT



Richard Hynes Ludwig MIT

Lung adenocarcinoma, an aggressive and common form of cancer, can arise from benign tumors known as adenomas. In a Nature paper published in May, a team led by Ludwig MIT investigator Tyler Jacks identified a switch that appears to nudge adenomas toward malignancy. Tyler and his team focused on the role of the Wnt signaling pathway in a mouse model of the cancer. Wnt plays an important role in the function of adult stem cells, and malfunctions in its signaling circuits have long been known to drive cancer. The researchers found that in adenomas, the Wnt pathway is silent. But as the benign tumors transition into a malignant mode, some 5 to 10% of their cells switch on the pathway, creating a pool of cells with cancer stem cell properties. Another 30 to 40%, meanwhile, create a niche for those cells by secreting the Wnt ligands themselves. A drug that interferes with Wnt ligand secretion significantly slowed tumor growth and extended the lives of mice by 50%. A survey of adenocarcinomas from patients showed that 70% harbored cells with Wnt activation, and 80% had the niche-building cells. All this suggests drugs that interfere with Wnt signaling might be effective treatments in the early stages of lung adenocarcinoma.

In another study, Tyler and his colleagues, including Ludwig MIT's own Richard Hynes, examined the protein composition of the extracellular matrix (ECM)-the complex meshwork of protein filaments and other large molecules that creates the scaffolding for cells in tissues, and helps regulate their growth and behavior. He and his colleagues quantitatively assayed the abundance of 113 ECM proteins in normal and fibrotic lung tissue, and in lung tumors and metastases to study the contribution of the tissue microenvironment to lung fibrosis and tumor progression. They report in a Proceedings of the National Academy of Sciences paper published in July the identification of specific protein signatures for fibrosis, primary tumors and metastases. One ECM marker of adenoacrcinoma, Tenascin-C (TNC), they showed, promotes lung cancer metastasis. They also report that levels of TNC and two other ECM proteins (S100A10 and S100A11) are strongly predictive of survival in patients with lung cancer. This signature could be used to improve diagnosis of adenocarcinoma and, with more study, to develop new treatments for the disease.

TO CATCH A KILLER

Ovarian cancer is a silent killer that begins with few signs and symptoms. Yet catching it early is crucial to improving a woman's odds of survival: When detected early, five-year survival rates can be greater than 90%. In an April paper in Nature Biomedical Engineering, a team led by Ludwig MIT scientist Sangeeta Bhatia reported a new technique to detect tiny ovarian cancer tumors-smaller than 2 millimeters in diameter-allowing for detection an estimated 5 months earlier than existing tests. The new test generates a synthetic biomarker-a nanoparticle that interacts with tumor proteins, issuing a readout that can be detected in urine samples.

This technique improves significantly on existing biomarker tests, which tend to generate very weak signals. It harnesses matrix metalloproteinases (MMPs), enzymes found in abundance within tumors, to generate a signal based on their activity. After being injected into a mouse, nanoparticles coated with peptides (which are cleaved differently by different MMPs) collect within the tumor. MMPs snip the peptides on their surface to liberate peptide fragments, which are then filtered out by the kidney and concentrated in the urine, where they can be detected using various methods, including a simple paperbased test.



Sangeeta Bhatia Ludwig MIT



Alexander Rudensky Ludwig MSK

NECESSARILY DIVERSE

Regulatory T (Treg) cells suppress inflammation and autoimmunity and are marked by their expression of a master regulator of gene expression, the transcription factor FoxP3. Oddly, however, many Tregs are also known to express transcription factors that enhance the activity of helper T cells, which promote inflammation and immune attack. Whether this expression is reversible or essential to the function of Treg cells has been open to question. In a June Nature paper, researchers led by Ludwig MSK Director Alexander (Sasha) Rudensky report their examination of the function of Treg cells expressing one of those factors, T-bet. They found that eliminating T-bet-expressing Treg cells but not T-bet

expression in Treg cells in mouse models resulted in severe autoimmunity driven by type-1 helper T cells (TH-1) and killer T cells (rather than by the type-2 helper T cells that promote an antibody response). On the other hand, when Treg cells that do not express T-bet were selectively depleted, the T-bet expressing Treg cells that remained specifically inhibited TH-1 cells and killer T cell activation, and were found in the company of T-bet-expressing target cells. The findings suggest that T-bet-expressing Treg cells play a distinct role in immunosuppression. They also indicate that Treg cells-like helper T cells-come in a variety of functional flavors, with each having a specific role in the processes that suppress autoimmunity.

CIRCLES OF RESISTANCE

In a groundbreaking study published in Nature in March, an interdisciplinary team of researchers led jointly by Ludwig San Diego's Paul Mischel and Vineet Bafna of the University of California at San Diego report that short circles of DNA harboring multiple copies of oncogenes are relatively widespread in cancer and appear to contribute significantly to cancer cell diversity and tumor evolution across a range of malignancies. The researchers analyzed cells from 17 different types of cancer and found such extrachromosomal DNA (ecDNA) in 40% of tumor cell lines but rarely in normal cells. When they looked specifically at patient-derived models of brain tumors, nearly 90% of these carried ecDNA. Unlike chromosomes, ecDNA is parceled out randomly to daughter cells when a tumor cell divides. It appears that this contributes greatly to the diversity of cancer cells in a tumor, and that diversity in turn enhances the ability of tumors to adapt to environmental challenges, including drug therapy. The research stems from a previous 2014 Science study led by Paul that revealed that ecDNA plays a central role in the drug resistance of the aggressive brain cancer glioblastoma multiforme.



Paul Mischel Ludwig San Diego



Bert Vogelstein Ludwig Johns Hopkins

VAGARIES OF CHANCE

The cell's DNA replicating machinery is stunningly accurate. But like every other machine in the universe, it is far from perfect and mutations that lead to cancer are often caused by inadvertent errors that occur as stem cells in various organs copy their DNA to divide. Researchers led by Ludwig John Hopkins Co-director Bert Vogelstein and his Hopkins colleague and mathematician Cristian Tomasetti set out to determine what proportion of cancer mutations stem from such errors, as opposed to inheritance and environmental factors, such as smoking and sunbathing. They reported in a March study in Science that their analysis of 32 types of cancer suggests that, overall, 66% of cancer mutations come from copying errors and 29% from lifestyle or environmental factors, while 5% are inherited. As might be expected, the results varied considerably between cancer types. About 77% of cancer-driving mutations in pancreatic tumors come from random copying errors, while 18% can be traced to environmental factors, such as smoking, and 5% are inherited. In lung cancer, on the other hand, 65% of such mutations are due to environmental factors-mostly smoking-while 35% arise from copying errors.

VULNERABILITY UNVEILED

There are no targeted treatment options available for women diagnosed with an aggressive form of breast cancer known as triple-negative breast cancer (TNBC). It accounts for nearly 20% of breast cancer cases and is defined by the absence of receptors for estrogen, progesterone and human epidermal growth factor (HER2) on its cells. Chemotherapy is used as a first line of treatment but most TNBC tumors don't respond well to such treatments, and those who do often develop resistance to the drugs. Now a team of researchers led by Alex Toker, an investigator at Ludwig Harvard, has discovered a TNBC vulnerability that could be exploited for therapy with existing drugs. Their findings were published in April in Cancer Discovery. They first treated TNBC cells with a

DNA-disrupting chemotherapy called doxorubicin. To compensate, the cancer cells increased production of pyrimidine nucleotides-molecular components of DNA-a response that accounts for resistance because it also boosts the cell's ability to repair its DNA. To undermine that resistance, they treated the TNBC cells with a combination of doxorubicin and leflunomide, a drug that compromises the production of pyrimidine nucleotides and is mainly used to treat rheumatoid arthritis. The treatment induced significant tumor regression in mice, and appeared to be well tolerated. Alex and his team hope to initiate clinical trials of the combination, and are exploring the underlying mechanisms of enhanced pyrimidine biosynthesis in TNBC cells.



Alex Toker Ludwig Harvard

MECHANISMS OF ESCAPE

A team of researchers including Ludwig Harvard Co-director George Demetri has reported an exceptionally responding patient with a metastatic mesenchymal cancer, and their analysis has exposed mechanisms of sensitivity and resistance to immune checkpoint blockade therapy. They report in a recent issue of *Immunity* their comparisons of tumors that were responsive to the therapy with a single resistant metastasis from this patient, who had metastatic uterine leiomyosarcoma-a rare malignancy that arises from the precursors of smooth muscle cells in the uterus. This patient had complete response of all sites of disease for more than two years when given anti-PD-1

therapy with pembrolizumab (which disrupts a mechanism tumor cells exploit to protect themselves from immune attack). After a single site resistant tumor grew, it was removed surgically, and the patient has maintained a complete response to therapy. George and his team found that the cells of the resistant tumor had low expression of two cancerrelated antigens that stimulate a brisk anti-tumor immune response as well as a lasting memory of their identity. Further, the tumor had lost both copies of the PTEN tumor suppressor gene. Functionally deleterious mutations in PTEN have been shown to confer resistance to checkpoint blockade in melanoma as well.



George Demetri Ludwig Harvard



Alexander Rudensky Ludwig MSK



Bert Vogelstein Ludwig Johns Hopkins



Ken Kinzler Ludwig Johns Hopkins



Nick Papadopoulos Ludwig Johns Hopkins

TOWARD PREVENTION

In February 2015, Ludwig and the Conrad N. Hilton Foundation renewed their partnership in cancer prevention, with each contributing \$5 million over five years to the effort.

The program, which focuses on colorectal cancers, has two overarching scientific goals. One is to explore and validate nutritional approaches for the prevention of colon cancers, and to ready them for clinical evaluation. To that end, Alexander (Sasha) Rudensky's lab at Ludwig MSK is exploring the role of suppressive immune cells known as regulatory T cells (Tregs) in colon cancer. His team recently published a paper in *Nature Immunology* exploring the role of the Interleukin-2 receptor in Treg function. Their findings also suggested that Tregs limit precancerous lesions in the early stages of disease, but promote cancerous growths in later stages.

The second research goal is to develop reliable, noninvasive DNA- and protein-based tests for the routine detection of precancerous growths and incipient cancers of the colon. This goal builds upon earlier accomplishments of the Ludwig Johns Hopkins team of Bert Vogelstein, Ken Kinzler and Nick Papadopoulos, in collaboration with Peter Gibbs and Jeanne Tie, formerly of Ludwig Melbourne. The team reported in a *Science Translational Medicine* paper last year the development and early evaluation of a circulating tumor DNA (ctDNA) test for recurrence of colon cancer in patients treated for stage II disease.

Three groups from Ludwig Oxford will now add to these efforts. One, led by Chunxiao Song, also utilizes ctDNA. In particular, Chunxiao's group has developed very sensitive and potentially low-cost methods to detect epigenetic modifications to such DNA. These modifications are chemical tags that help control gene expression and are widely redistributed in cancer. In a proof of principle study, he and his colleagues showed that epigenetic information in ctDNA varies by cancer type (including colorectal cancers) and could be used to discern such things as its tissue of origin and even the stage of the tumor from which it came.

Another group, led by Gareth Bond, will be looking at how natural variations in DNA sequence known as singlenucleotide polymorphisms (SNPs) influence the risk of colon cancer in the presence or absence of mutations in cellular signaling circuits that are known to drive the disease. Gareth and his team have already done a pilot study with hundreds of patients looking at SNPs that appear to influence such risk in the presence or absence of mutations to p53, a tumor suppressor known as the "guardian of the genome" whose activity is commonly



Peter Gibbs Ludwig Melbourne alum



Jeanne Tie Ludwig Melbourne alum



Chunxiao Song Ludwig Oxford



Gareth Bond Ludwig Oxford

disrupted across cancer types. They found 13 such SNPs in the study—all linked to the normal p53 gene—that associate with large differences in cancer risk. They are now expanding their search to look for SNPs with respect to four other major cancer-related signaling pathways that are also the targets of therapies. The identified SNPs could help improve screening and strategies and increase the likelihood of detecting cancers at an early stage, when curative interventions are possible.

Finally, a group led by Skirmantas Kriaucionis proposes to look at whether epigenetically modified nucleosides (the bases in DNA that encode information) in the diet boost the risk of developing colorectal cancers. Studies in his lab suggest that high doses of such nucleosides can damage DNA, and so induce mutations. Skirmantas will measure the abundance of these bases in common foods and the bugs that inhabit our intestines, test whether they promote colon cancer in a mouse model prone to the disease and see if he and his team can't identify DNA signatures associated with such mutations, if they do occur. This could lay the groundwork for epidemiological studies identifying diets that might influence cancer risk through such mechanisms.

On the policy front, the Initiative will begin to address a third component of its overall agenda—informing and influencing public policies for cancer prevention. To that



Skirmantas Kriaucionis Ludwig Oxford

end, Ludwig's Deputy Scientific Director Bob Strausberg and Vice President for Communications Rachel Steinhardt, together with Ludwig partners including Cancer Research UK, will convene a working group to prepare for an international meeting on the topic to be held in late 2018 that includes scientists and public health experts from around the world.

"We are excited by this opportunity to assemble a worldclass interdisciplinary team to discuss and put forth a recommended framework to tackle the biggest obstacles in the study of nutrition-based approaches to cancer prevention research," said Bob.

Clinical trials

FISHING FOR BAIT

A team led by Ludwig Stanford scientist Ash Alizadeh and colleagues has shown how to identify, for therapeutic use, the neoantigens that reveal to the immune system the presence of human mantle-cell lymphoma (MCL), a type of B-cell non-Hodgkin's lymphoma (NHL). Neoantigens are protein fragments-or peptides-derived from mutated genes that are presented to the immune system by cells to reveal the presence of a cancer. The presenting is done by a family of cell-surface proteins known as the major histocompatibility complex (MHC). Ash and his colleagues conducted an analysis of tumor neoantigens presented by the MHC in 17 MCL patients. They reported in March in Nature that every one of the neoantigen peptides recognized by the immune system was a snippet of a variable region of an antibody. (These genes are expressed by B cells, which secrete antibodies and from which MCLs arise.) Oddly, none came from the cell's thousands of other genes. Almost all of these neoantigenic peptides were presented to the immune system by a class of MHC that specifically activates helper T cells. These are orchestrators of immune responses that, in turn, activate killer T cells, macrophages and B cells to seek and destroy bearers of targeted antigens. Ash and his team show that the helper T cells they captured mediate the killing of MCL cells. They argue that their approach can be used to find tumor neoantigens in patients and devise novel immunotherapies for the treatment of MCL.



Ash Alizadeh Ludwig Stanford

BREAKING THROUGH CANCER'S SHIELD

In April, the immune-oncology company Agenus began testing a new therapy discovered in collaboration with Ludwig, in cervical cancer and solid tumors. The Phase 1/2 clinical trial is designed to evaluate the safety and pharmacological activity of the new PD-1 inhibitor AGEN2034 in patients with solid tumors. PD-1 is expressed on the surface of immune cells and allows tumors to fly below the radar of the immune system. Blocking PD-1 can revive T cell attack of cancer cells and, in many patients, induces significant therapeutic responses. The study's primary goal is to assess the dose-limiting toxicity of the drug, the maximum tolerated dose and the best overall response. Phase 1 of the trial will assess the safety, tolerability, and clinical activity of ascending doses in patients with metastatic or locally advanced solid tumors. Each dose cohort will include three patients. The Phase 2 part of the study will expand to include patients with recurrent, unresectable or metastatic cervical cancer whose disease continued to progress after treatment with a two-drug combination. AGEN2034 was originally developed under a collaborative research and development agreement between Ludwig Cancer Research, Agenus and Ludwig start-up Recepta Biopharma S.A., which holds the development rights to AGEN2034 in Brazil and other South American countries.





BOB STRAUSBERG LUDWIG'S DEPUTY SCIENTIFIC DIRECTOR

How did you get into science?

I have always loved science. I would go off with my two younger sisters to the bookstore and wander into the science section and buy books on biology. I was absolutely fascinated by nature as a problem-solver. I love genetics, too, because there is such preciseness to it. I was the first one in my family to get a PhD and build a scientific career. I've always pursued things in my heart that just felt right and I've never regretted the decision to pursue science. And I'm still inspired by it every day because I chose to do what I love to do.

Tell us about your early career.

When I started my career, things were very different. Many of the tools of modern biology had not yet been developed. What we tended to do was work on simpler

systems. I worked on baker's yeast, a much simpler system but one that shares many characteristics with cells of more complex organisms, including humans. I got my PhD, did my postdoc and then became an assistant professor of biology at Southern Methodist University, in Dallas, Texas, and continued to pursue my research. I enjoyed teaching, and had great undergrad students. But then I made this abrupt switch from an academic career to joining a company called Genex Corporation. I became engaged in projects where we were trying to develop vaccines, biomaterials, and therapies using yeast. There I learned that my platform had many practical applications and this allowed me to pursue a diversity of biology, which is really my interest. I also learned about the remarkable benefits of team-based science. In looking back at my career, it's always been about collaboration

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The experience helped me realize that if you choose a certain career path, it doesn't mean you're stuck on that path forever.

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and interdisciplinary science, which I find very invigorating. You benefit from many people's knowledge and can be part of a big common goal.

How did you get into cancer research?

My entry into cancer research was an unusual one. When I was at Genex, people who knew me were starting the Human Genome Project, and they were interested in having me come to it because I had some of the technical knowledge that would be required, and I knew how to work with industry. They wanted somebody who could bring together teams of people from academia and industry. That's how I started working on the Human Genome Project, developing sequencing technologies. I learned a lot about doing team science on a truly international scale with the best scientists in the world. It's from there that I entered cancer research because, at that time, people were just beginning to think about cancer genomics. With that, I had the opportunity to meet the director of the National Cancer Institute (NCI) at the time, who was Richard Klausner, and he asked if I'd work directly with him and start the cancer genomics program for the NCI. So I didn't start in cancer research as a scientist

beginning a career, as is usually the case, but by being thrown onto one of the biggest platforms in the world, working with the director of the NCI, on what was probably the most visible program at the NCI back then.

Looking back at your career now, what is the most surprising decision you made?

Going to work for a biotech was the real surprising decision. There was nothing in my academic training that would have said to do that. I always thought that prestige came from having an academic career. I like basic biology but I really like to be a problem solver, to do something that has more direct applications. I was thinking about this at a time when the big biotechs, like Genentech and Biogen, were just starting, and the genes for human growth hormone and insulin had been cloned. Now, I was pretty shy, and people would never have guessed I'd make these career leaps but, surprisingly, I did. The experience helped me realize that if you choose a certain career path, it doesn't mean you're stuck on that path forever.

What are you most proud of in your career so far?

There have been quite a few good moments but I think it would probably have to be when I was given responsibility for the Cancer Genome Anatomy Project that was created by the NCI. It was pretty thrilling to be standing next to former Vice President AI Gore at the press conference when he introduced the project. Basically, we generated a wide range of genomics data on cancerous cells accessible through easy-to-use online tools. Researchers, educators

Q&A

and students were able to find answers in silico to biological questions through the website. Characterizing cancer at a molecular level with readily accessible and up-to-date data and a new set of tools was a completely different way of doing science. I think it actually inspired the next generation with what's going on now with big science and big data.

What are your favorite hobbies or interests?

I love music and going to concerts to enjoy it live. I'm interested in all sorts of music. I like going with the flow and learning about new things, and when it comes to music, this has helped me build relationships as I was growing up and, later, with my children. When I was in college, living in New Jersey, my friend and I saw this poster one weekend for a festival in Woodstock. We didn't know what it was all about, but we went and bought a ticket, and drove up there in a Rambler with a big peace symbol on the back. I have always been open to different genres. My son's very interested in all kinds of alternative music, so I've spent a fair amount of time in recent years going to ska concerts and punk rock festivals. I also like Broadway musicals. I admire the creativity of musicians, and it reminds me of the creativity of scientists. Many kinds of music are collaborations and require teamwork, and everything comes together to make something way different from what you'd get with a solo musician.

What do you see as the major barriers to progress in cancer research?

Science and technology are on fastforward but we're not connecting all the dots with each scientific advance. We're



not doing a good enough job of asking, what are the real lessons learned? Who can we bring together? Who should be working together? Our real mission is to help cancer patients, but we can do a better job of building connectivity among our teams of scientists and their research to make more of an impact in that way. We need to put the idea of team science-collaborative, interdisciplinary research, which has become central to scientific discovery-into practice. Team science is great in theory but so hard in practice. Another cultural problem is that researchers are-understandablyreluctant to take risks. A lot of research one sees is only slightly different but mainly 'me too.' One of the things I think about is, what are the bold steps we can take? Can we do team based science that will really have a big impact? It may take a long time, but it can also pay off in a big way. That was one of the things that attracted me to Ludwig, which has a history of doing that kind of thing, of taking big steps.

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What do you see as the most promising areas of cancer research today?

Immunotherapy has had a huge impact. I feel like cancer meetings today are way different than they were just four years ago. Back then, all the talk was about small molecules targeting a gene, and there was this sense that this is still going to be very difficult, and every drug is going to get resistance. Then immunotherapy came along, and while there is appropriate caution about claiming cures, physicians and researchers are talking about durable responses and the fact is there are people now living who, a few years ago, would have died. Not everybody benefits, but it has changed the thinking a little from, 'every cancer is different and we have to have a designer approach for each type', to maybe doing things with immunotherapy that can have an impact on a broad range of cancers. The other major interest of mine has been a stepchild in the field for a long time: how we prevent cancer. We know from other diseases that prevention is the most effective approach. The biggest advances have been things like clean drinking water and vaccines. May be we can come up with simple solutions that will help people not just here but in Asia, Africa and Latin America as well.

What is Ludwig doing in the area of cancer prevention?

Ludwig launched a research program in partnership with the Hilton Foundation to advance dietary interventions and technologies for the prevention of colon cancer [see page 14]. Researchers from Ludwig Johns Hopkins, Ludwig MSK and Ludwig Oxford are bringing their expertise in a range of disciplines critical to this program. We've developed strong partnerships with Cancer Research UK and the City of Hope and conducted a major outreach to other organizations that are also involved in the area of prevention, like the World Health Organization, the Wellcome Trust and the Medical Research Council UK. My role is to facilitate this program and provide context about what we are trying to accomplish. Our goal is to bring major researchers to this important area, and also provide leadership and partnership with organizations and researchers throughout the world.

What excites you about the future of Ludwig?

If you look at Ludwig, we're not one of the biggest cancer organizations in the world, but we're privileged in that we do have resources to work with. We don't have to go out and raise new funds for everything we want to do. We can and should be risk-takers. That's what has always excited me about Ludwig. We don't have to do what everybody else is doing. So we need to think smart and maximize our impact with the funds we have, doing things that even the NCI might not do with the billions of dollars at their disposal each year. What excites me is we can think in a bold way and bring the best scientists to a problem and support them for the longterm. I like that even though our scientists publish a lot of good papers, we don't measure them solely on that basis. We are committed to making a difference for people. That's what brought me to Ludwig. I'm excited we have a new scientific director who will help us take a fresh look at where Ludwig has been, where we are

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now, and make sure we are pursuing the bold mission that we really should be undertaking.

You are one of the few leaders of this organization who has started to use social media more actively. What are the benefits of being engaged?

I didn't grow up with social media but I've come to appreciate many aspects of it, especially in building connections and community. My Twitter account has become an important information source for me and has helped me select a community of people who share my research interests. I value the real-time interaction of Twitter and when I go onto the Ludwig feed and retweet something I like, I'm also able to see who else is retweeting it, reinforcing that sense of community. We hear about important papers on Twitter as soon as they are released, or even before, so tweeting allows us to communicate actual science and not just news.

Who are the scientists, living or dead, that you admire?

I've been very fortunate to have worked with many visionary scientists. In particular I would mention Lloyd Old who had a very big impact on me. He was a true gentleman and a very caring man. He was an unconventional visionary who pursued an area of science—tumor immunology—that was not a very popular avenue of research. But he never gave up, as he truly believed in the power of the immune response to fight cancer. Lloyd enriched my life as he did so many others and I'm better off for having known him.



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Ask a scientist

What has been the greatest challenge in the sequence analysis of the cancer genome?



As it is for early detection of cancer, the greatest challenge is to differentiate true mutations from artificial errors in a highly efficient, accurate and, most importantly, cost-effective way. I am optimistic and look forward, to that end, to seeing great improvements in the near future in sample manipulation, machine platforms and variant-calling algorithms.

MING ZHANG Ludwig Johns Hopkins



The greatest technical challenge we face is tumor heterogeneity; biologically, it is the age-old enigma of linking genotype to phenotype. Fortunately, dramatic advances in the affordability, quality and availability of genetic, epigenetic and RNA sequencing methods have begun to address these issues—with a goal of identifying personalized cancer treatments.

MICHAEL McCLELLAN Ludwig Oxford



Whole genome sequencing has been crucial in characterizing genomic aberrations in cancer. However, initial experiments were performed on bulk samples and overlook the heterogeneity of tumors. This is being overcome by single-cell genomics, which can help deconvolute tumors, leading to an improved understanding of tumor etiology and personalized treatment options.

RAMYA RAVIRAM Ludwig San Diego

Required reading

Ludwig Harvard

Science Translational Medicine 2017 May 24

The brain microenvironment mediates resistance in luminal breast cancer to PI3K inhibition through HER3 activation.

Kodack DP, Askoxylakis V, Ferraro GB, Sheng Q, Badeaux M, Goel S, Qi X, Shankaraiah R, Cao ZA, Ramjiawan RR, Bezwada D1, Patel B, Song Y, Costa C, Naxerova K, Wong CSF, Kloepper J, Das R, Tam A, Tanboon J, Duda DG, Miller CR, Siegel MB, Anders CK, Sanders M, Estrada MV, Schlegel R, Arteaga CL, Brachtel E, Huang A, Fukumura D, Engelman JA, Jain RK.

Cancer Discovery 2017 April

Adaptive reprogramming of de novo pyrimidine synthesis is a metabolic vulnerability in triplenegative breast cancer.

Brown KK, Spinelli JB, Asara JM, Toker A.

Immunity 2017 February 21 Loss of PTEN is associated with resistance to anti-PD-1 checkpoint blockade

therapy in metastatic uterine leiomyosarcoma. George S, Miao D, Demetri GD, Adeegbe D, Rodig SJ, Shukla S, Liacebitz M, Amin Manaour A, Dai

Lipschitz M, Amin-Mansour A, Raut CP, Carter SL, Hammerman P, Freeman GJ, Wu CJ, Ott PA, Wong KK, Van Allen EM.

Ludwig Johns Hopkins

Science 2017 March 24 Stem cell divisions, somatic mutations, cancer etiology, and cancer prevention.

Tomasetti C, Li L, Vogelstein B.

Ludwig MIT

Proceedings of the National Academy of Sciences U S A 2017 July 11 Quantitative proteomics identify

Tenascin-C as a promoter of lung cancer progression and contributor to a signature prognostic of patient survival.

Gocheva V, Naba A, Bhutkar A, Guardia T, Miller KM, Li CM, Dayton TL, Sanchez-Rivera FJ, Kim-Kiselak C, Jailkhani N, Winslow MM, Del Rosario A, Hynes RO, Jacks T.

Nature 2017 May 18

A Wnt-producing niche drives proliferative potential and progression in lung adenocarcinoma.

Tammela T, Sanchez-Rivera FJ, Cetinbas NM, Wu K, Joshi NS, Helenius K, Park Y, Azimi R, Kerper NR, Wesselhoeft RA, Gu X, Schmidt L, Cornwall-Brady M, Yilmaz ÖH, Xue W, Katajisto P, Bhutkar A, Jacks T.

Nature Biomedical Engineering 2017 April 10 Ultrasensitive tumourpenetrating nanosensors

of protease activity.

Kwon EJ, Dudani JS, Bhatia SN.

Ludwig MSK

Nature 2017 June 15 Stability and function of regulatory T cells expressing the transcription factor T-bet.

Levine AG, Medoza A, Hemmers S, Moltedo B, Niec RE, Schizas M, Hoyos BE, Putintseva EV, Chaudhry A, Dikiy S, Fujisawa S, Chudakov DM, Treuting PM, Rudensky AY.

Ludwig San Diego

fates.

Genes & Development 2017 July 11 [Epub ahead of print] Kinetochores accelerate or delay APC/C activation by directing Cdc20 to opposing

Kim T, Lara-Gonzalez P, Prevo B, Meitinger F, Cheerambathur DK, Oegema K, Desai A. Molecular Cell 2017 July 6 mTORC2 regulates amino acid metabolism in cancer by phosphorylation of the cystineglutamate antiporter xCT.

Gu Y, Albuquerque CP, Braas D, Zhang W, Villa GR, Bi J, Ikegami S, Masui K, Gini B, Yang H, Gahman TC, Shiau AK, Cloughesy TF, Christofk HR, Zhou H, Guan KL, Mischel PS.

Developmental Cell 2017 May 22 Dephosphorylation of the Ndc80 tail stabilizes kinetochoremicrotubule attachments via the Ska complex.

Cheerambathur DK, Prevo B, Hattersley N, Lewellyn L, Corbett KD, Oegema K, Desai A.

Nature Communications 2017 May 12

PTEN regulates glioblastoma oncogenesis through chromatinassociated complexes of DAXX and histone H3.3.

Benitez JA, Ma J, D'Antonio M, Boyer A, Camargo MF, Zanca C, Kelly S, Khodadadi-Jamayran A, Jameson NM, Andersen M, Miletic H, Saberi S, Frazer KA, Cavenee WK, Furnari FB.

Nature Methods 2017 June A tiling-deletion-based genetic screen for cis-regulatory element identification in mammalian cells.

Diao Y, Fang R, Li B, Meng Z, Yu J, Qiu Y, Lin KC, Huang H, Liu T, Marina RJ, Jung I, Shen Y, Guan KL, Ren B.

Nature 2017 March 2 Extrachromosomal oncogene amplification drives tumour

evolution and genetic heterogeneity.

Turner KM, Deshpande V, Beyter D, Koga T, Rusert J, Lee C, Li B, Arden K, Ren B, Nathanson DA, Kornblum HI, Taylor MD, Kaushal S, Cavenee WK, Wechsler-Reya R, Furnari FB, Vandenberg SR, Rao PN, Wahl GM, Bafna V, Mischel PS.

Ludwig Stanford

Nature Communications 2017 May 31

Systematic discovery of mutation-specific synthetic lethals by mining pan-cancer human primary tumor data.

Sinha S, Thomas D, Chan S, Gao Y, Brunen D, Torabi D, Reinisch A, Hernandez D, Chan A, Rankin EB, Bernards R, Majeti R, Dill DL.

Nature 2017 May 25

PD-1 expression by tumourassociated macrophages inhibits phagocytosis and tumour immunity.

Gordon SR, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, Gupta R, Tsai JM, Sinha R, Corey D, Ring AM, Connolly AJ, Weissman IL.

Nature 2017 March 30 Antigen presentation profiling reveals recognition of lymphoma immunoglobulin neoantigens.

Khodadoust MS, Olsson N, Wagar LE, Haabeth OA, Chen B, Swaminathan K, Rawson K, Liu CL, Steiner D, Lund P, Rao S, Zhang L, Marceau C, Stehr H, Newman AM, Czerwinski DK, Carlton VE, Moorhead M, Faham M, Kohrt HE, Carette J, Green MR, Davis MM, Levy R, Elias JE, Alizadeh AA.

Ludwig Stockholm Genes & Development 2017 May 15

Neuroblast differentiation during development and in neuroblastoma requires KIF1B mediated transport of TRKA.

Fell SM, Li S, Wallis K, Kock A, Surova O, Rraklli V, Höfig CS, Li W, Mittag J, Henriksson MA, Kenchappa RS, Holmberg J, Kogner P, Schlisio S.



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