2015 RESEARCH HIGHLIGHTS

LIFE-CHANGING SCIENCE





WELCOME

Ludwig Cancer Research has, since its earliest days, injected resources into every phase of the cancer research continuum—from the basic science that unravels the fundamental processes of life to the applied research essential to the design and development of new drugs and diagnostics. What distinguishes our approach, however, is that we have just as consistently sought to invest in the people behind the science as much as in the science itself. We do this by providing Ludwig's researchers with steady, long-term funding, which is a rarity these days. It means that our scientists get more time and latitude than most to develop hypotheses, to take considered risks, to refine and test their ideas.

We think the approach works rather well. It has over the past few decades helped Ludwig researchers make landmark contributions to cancer research, illuminating the darkest corners of human biology and opening new doors to the treatment of cancer-perhaps most notably immunotherapy. In many cases, Ludwig has helped its researchers convert their insights into novel diagnostics and therapies.

This past year was no exception. So we've tried in this Research Highlights Report to not only describe the most captivating discoveries made with Ludwig's support in 2015, but to introduce you to the people behind those discoveries as well. In the pages that follow, you will learn a good deal about how their work has created new possibilities for cancer patients. But we hope you will get something else as well: a glimpse of the personal journeys, professional fascinations, friendships and partnerships that were instrumental to their discoveries.

Still, there's no shortage of science in this report, which touches on topics ranging from the regulation of the cancer genome to the adaptability of cancer cells to their susceptibility to immunotherapy. Beyond that, the stories here are, collectively, something of a testament to the power of collaboration. They reveal how colleagues—and, in some cases, old friends—across Ludwig put their heads together to solve tough problems and find new solutions to the many conundrums of cancer.

In 2015, such partnerships resulted in powerful new methods to parse the cellular contents of tumors, test immunotherapies in combination and, in one case, solve a century-old mystery of cell biology. You will also learn here about Ludwig's own collaboration with the Cancer Research Institute—how and why it was forged and what it is doing to advance the clinical assessment of novel immunotherapeutic strategies for the treatment of cancer.

We hope you enjoy this report. Happy reading!

Sincerely,

Ed and David

Edward a. Mengemin



Edward A. McDermott Jr. President and Chief Executive Officer

David Long



Sir David Lane, PhD Scientific Director



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MAKING DISCOVERIES





"Gene regulation is such a fundamental problem in biology."

BING REN

GENOME'S TOPOGRAPHER

Bing Ren took his first real stab at grown-up science in the early 1980s. A strapping middle-schooler in Taiyuan, the capital of China's coal-rich province of Shanxi, Ren got word that NASA was soliciting suggestions for experiments that might usefully be conducted in orbit. Excited by the possibility—his brainchild, in outer space!—Ren in short order mailed out his very first research proposal to the authorities. "It was not selected," he recalls.

If outer space once disappointed, Ren has had much better luck with the inner variety: His scientific forays into the cell's nucleus are illuminating how the genome controls its own expression—and how that control runs awry in diseases such as cancer. Thanks to his experimental virtuosity, Ren has helped launch a revolution in genomics, one that has made him, according to Thomson Reuters, among the most influential researchers in his field.

In a series of papers published in 2015, Ren and his team at Ludwig San Diego captured on a vast scale the variability of the genome's expression through the early stages of development and across an array of cell types, linking that variation to the chemical modification and physical structure of chromosomes; they worked with other Ludwig researchers to profile in vivid detail how aberrant signals from a mutant receptor alter the activation of the genome in cells of the brain cancer glioblastoma multiforme (GBM); and they partnered with another group of scientists to describe how stem cell chromosomes sequentially unfurl as they prepare to generate cells of the pancreas and liver.

First steps

Ren's journey to the frontiers of genomics began at the University of Science and Technology of China, where he majored in biophysics, studying the neurology of visual processing. But soon after he started his doctoral studies at Harvard in 1992, he became fascinated by gene regulation and joined the laboratory of Tom Maniatis, one of the pioneers of modern molecular cloning. "Gene regulation is such a fundamental problem in biology," says Ren. "It explains how all these different types of cells in the body emerge from the information encoded by the genome."

Ren focused on how specialized proteins called transcription factors control gene expression by recognizing unique DNA sequences. As was the norm at the time, he probed such interactions one at a time, outside the cell, binding the protein factors to their target DNA sequences in a test tube. But Ren was eager to capture such events as they occur inside cells and, a few years into his research, adopted a technique to do so. It involved chemically gluing the proteins to their target DNA inside the cell and then using antibodies to pull the whole scrum down for subsequent analysis.

After obtaining his PhD, Ren joined Richard Young's laboratory in the Whitehead Institute at MIT as a postdoctoral researcher. Young's team was at the time using DNA microarrays–glass slides peppered with short DNA sequences—to fish out the full spectrum of genes expressed by cells in response to various stimuli. Ren wanted to similarly profile the DNA switches that control such gene expression.

To that end, he adapted his antibodybased assay to the DNA chip, developing a technology in 2000 that later came to be known as ChIP-chip. "It was the first technique that permitted the large-scale analysis of the genetic switches responsible for gene expression," says Ren.

As DNA sequencing technology evolved, Ren and other researchers further adapted his ChIP protocol to create ChIP-Seq, which is compatible with modern sequencing machines. This technology, and his laboratory's mastery of computational biology, have since powered Ren's prolific exposition of the genome's regulation and turned his Ludwig-supported laboratory into a technological engine for a new era of genomics.

The layered genome

If the human genome is a recipe book, its chapters are 23 distinct chromosomes, each of which is stuffed, in rough duplicate, into the nucleus of almost all the cells of the human body. But how is that single book read to build the body's diverse constituency of cells? Or, for that matter, to generate such a variety of humans? And how is it read differently by malfunctioning or cancerous cells?

In 2015, Ren made significant contributions to solving each of these problems as leader of two studies and senior author on a third. The papers were part of a package of six papers published in *Nature* summing up the findings of the \$300 million Roadmap Epigenomics Program. An initiative of the U.S. National Institutes of Health, the project had explored how chromatin—DNA and its protein scaffolding—is chemically tagged to control gene expression.

Such "epigenetic" tags have long been known to help control gene expression and to be broadly misplaced in cancer cells. Stretches of chromatin that are tagged to be silent are typically bundled up and so sequestered from the cell's gene-reading machinery. Those bearing genes to be expressed are, conversely, held open and available. These patterns give resting chromosomes a subtle and layered structure that is directly related to gene expression.

One of Ren's *Nature* studies employed ChIP-Seq to determine the degree to which the same genes—known as alleles—inherited from each parent are differently expressed across the genome. It tied that difference in expression to the distribution and sequence of "enhancer" DNA sequences, which boost the expression of specific genes. Ren and his colleagues found that roughly 30 percent of the gene set we carry is expressed variably between the two copies in some of 20 tissues and cell types examined. Much of that variation appears to be due to differences in DNA sequences of enhancers and other regulatory sequences of DNA.

His other *Nature* study examined how the 3D structure of chromosomes and their epigenetic landscapes differ between different types of adult and embryonic cells. It also integrated data from the former paper to reveal how all of these phenomena interact to ensure the appropriate expression of the genome. The ample and freely available data from these studies will for years be mined by researchers studying virtually every subfield of human biology, not least cancer.

Beyond basic biology

In two other studies published in 2015, Ren and his team took on the epigenetics of disease and the biological effects of chromosomal architecture. In one study, published in Molecular Cell, Ren partnered with Ludwig's Paul Mischel and Andrew Shiau to examine how a mutant growth factor receptor (EGFRvIII) that drives many GBM tumors alters the epigenetic landscape of the genome (see accompanying story, page 35). The team identified a large set of enhancer sequences that are aberrantly activated by the redistribution of epigenetic tags. The scientists then showed how two of the proteins produced at higher levels by such activation play a critical role in the survival of GBM tumors and used these findings to devise a potentially novel approach to treating GBM.

"This study is proof of principle that by analyzing noncoding, gene-regulating DNA sequences, we can get to the heart of the problem in a given cancer and identify new strategies for its treatment," says Ren.



Bing Ren Ludwig San Diego

For the other study, published in *Cell Stem Cell*, Ren partnered with a colleague at the University of California, San Diego, to detail how the sequential and fastidiously choreographed unfurling of chromatin is essential to the generation of pancreatic and liver tissues from stem cells. The findings have biomedical relevance because dysfunctions in the choreography of chromosomes might cause diseases like diabetes. The findings could ultimately also help researchers figure out how to make therapeutically useful tissues from stem cells.

Ren, it appears, is only getting started. His lab was recently picked as one of the technological hubs within a potentially \$120 million project named the 4D Nucleome Program. The project will chart over the next five years the relationship between the epigenetic control of gene expression and the three-dimensional structure of chromosomes—and how the two change over time, the eponymous 4th dimension.

If history is any guide, this project too will likely be of lasting importance to every subfield of the biomedical sciences. "Are [centrosomes] some sort of driving force in the genesis of tumors?"

KAREN OEGEMA, ANDREW SHIAU AND TIMOTHY GAHMAN

PUZZLERS AND DRUG DESIGNERS

Peering into fertilized roundworm eggs in the late 19th century, the legendary German zoologist Theodor Boveri identified a minute, beadlike structure associated with spindles that yanked chromosomes apart in the dividing cells of the embryos. Boveri named this "special organ of cell division" the centrosome and, over the next three decades or so, never quite took his eye off the structure. In 1914, a year before his death, he formally hypothesized that multiple copies of centrosomes fueled malignancy by inducing the accumulation of abnormal numbers of chromosomes in cancer cells.

Since then, researchers have parsed the structure and biochemistry of centrosomes and elucidated their many vital contributions to the cell's inner life. But it has remained unclear whether rapidly dividing cancer cells are indeed "addicted" to multiple copies of centrosomes. Ditto, oddly enough, for whether they're even needed for the proliferation of normal cells. In 2015, a full century and a year after Boveri published his hypothesis, a collaborative study led by Ludwig San Diego's Karen Oegema, and Andrew Shiau and Timothy Gahman of the Small Molecule Discovery Program, finally answered both questions. Their paper, published in Science, may have opened the door to implementing a strategy for treating

cancer fielded some two decades ago by Ludwig's scientific director, David Lane.

The puzzlers

When Oegema talks about the cell, or all of the things she can do with her microscopes, she sounds a lot like a kid in a candy store. Only, the treats in her case are some of the central problems of cell biology. "I have commitment issues," she jokes. "We wind up working on a lot of different projects in my lab."

Today, these include probing how dividing cells split in two, and using a spectacular, advanced high-content microscopy system obtained by Ludwig to chart out, on a grand scale in the flatworm *C. elegans*, what it is that the proteins encoded by all the genes involved in cell division and embryonic development exactly *do*.

But like Boveri, Oegema has a bit of a soft spot for the centrosome, whose biochemistry she studied for her doctoral research in the mid-1990s at the University of California, San Francisco (UCSF). "Centrosomes," she recalls, "were held up as a sort of Holy Grail in cell biology. It was very difficult to crack what their components do, define the scope of their role in cells and to describe precisely how they duplicate."

Oegema's Science paper stemmed from her work dissecting centrosome duplication in *C. elegans*—echoed by discoveries others had made in mammalian cells—which suggested that a protein known as Plk4 controls the assembly of centrioles, barrellike structures from which centrosomes are made. Oegema was interested in blocking this protein as a route to asking a range of basic biological questions.

Enter Shiau, who has known Oegema and her husband, Ludwig's Arshad Desai, since graduate school at UCSF and now heads Ludwig's Small Molecule Discovery Program. He and Gahman, who is a medicinal chemist, both worked for years in the biotech industry. They have a wealth of experience in the design and development of drug-like molecules for research and therapy and support Ludwig researchers around the world in this capacity.

"We look for problems like this, where investigators have a committed interest in a pathway or a problem," says Shiau. "Then Tim and I ask, is there a fundamental problem in cancer biology that can be answered?"

Teaming up

Oegema's proposal, they thought, fit the bill. "The centrosome question was very much on people's minds," says Oegema. "For a hundred years people have known that cancer cells have too many. The question is, why? Are they some sort of driving force in the genesis of tumors?"

The cell's single resident centrosome serves as an organizing center for its cytoskeleton, an intricate network of protein filaments that, among other things, confer shape, internal organization and motility upon cells. When a cell divides, though, the centrosome takes on its most famous function. It duplicates and helps ensure the equal distribution of chromosomes to the two daughter cells.

Biologists have, however, long known that other mechanisms exist to pull chromosomes apart, and many researchers once believed that centrosomes may not be required for cell division. On the other hand, it was clear that multiple centrosomes do contribute to the misdistribution of chromosomes seen in cancer cells.

To solve these mysteries, researchers had long sought to remove centrosomes by surgically excising them from cells or blasting them with lasers. But both normal and cancer cells treated this way simply remade their lost centrosomes and then continued dividing. Groups in academia and industry have tried to develop Plk4 inhibitors to target centrosomes. These work in the test tube shutting Plk4 down—but, for a variety of reasons, couldn't be used to stop cells from remaking their centrosomes.

To get around this limitation, the researchers designed and then tested hundreds of inhibitors for their effects on centrosomes in living cells. "We didn't care if we had a powerful inhibitor of Plk4 in the test tube," says Shiau. "What we cared about was getting a molecule that does what we want it to do inside the cell. You get what you screen for."



Timothy Gahman Ludwig San Diego

It took a while, but the team finally came up with a molecule that specifically and this was crucial to the subsequent study reversibly inhibited Plk4.

They showed that exposure to the molecule, which they named centrinone, eliminates centrosomes from both healthy and cancerous cells. Normal cells treated with centrinone simply stopped dividing and went into a state of latency known as arrest. Cancer cells, on the other hand, did not stop dividing when their centrosomes were removed, though fewer survived the ordeal.

"What we learned using centrinone is that normal cells do really care about having centrosomes and have the ability to detect their loss," says Oegema. Conversely, cancer cells are not addicted to multiple centrosomes. In fact, they continue dividing, albeit less efficiently, even when they have none.

History, again

The researchers showed that the pause in the division of healthy cells is governed by a tumor suppressor named p53. Dubbed the Guardian of the Genome, p53 is mutated in about half of all cancers. A couple of decades ago, Lane—a co-discoverer of p53—suggested that its role in stopping cell division in response to trouble of all sorts might be exploited for cancer therapy.

"The idea," says Shiau, "is that you trigger p53 in normal cells and have them stop multiplying—and then introduce another agent that only kills continuously dividing cells." Though centrinone is not a drug, molecules related to it may enable the experimental assessment of Lane's idea, which he named cyclotherapy. The new ability to reversibly eliminate centrosomes is also likely to benefit research in a wide variety of biomedical fields, given the organelle's multiple roles, from organizing the cytoskeleton to sprouting hair-like structures known as cilia on certain cells.

"Centrinone is a very good research tool, and we have been giving it out freely to academics and nonprofits," says Gahman. "Our next step is to ask what centrosome removal will do in animal models of cancer, and so we've made better, more drug-like molecules. It's one thing to look in a dish and see centrosomes go away. It can be quite a different thing when you're in a live tumor, in a living animal. That's where we're going now." "We both try to integrate our lab work and clinical work as much as possible."

M-LOT

ASH ALIZADEH AND MAXIMILIAN DIEHN

DNA DETECTIVES

There's no single formula for the elements of a productive partnership. But, as Ash Alizadeh and Maximilian Diehn would attest, a warm friendship certainly improves the chemistry. The Ludwig Stanford researchers have been fast friends for some 20 years now. "We're basically family," says Alizadeh. "We started medical school in Stanford at the same time. We were in graduate school together. We still work on the same lab bench, sharing reagents, food and all the stuff we did at graduate school. Our offices are right next to each other and we run joint group meetings. We're connected in almost everything we do."

That includes some rather brilliant science. In 2015, their extended collaboration resulted in the publication of two particularly noteworthy papers. First, their laboratories described in *Nature Methods* an analytical process named Cibersort that applies gene expression profiling and advanced computational analysis to trace back the precise spectrum of cell types contained in a slurry of disassembled tissue.

In the second study, published in *Nature Medicine*, Alizadeh, Diehn and their colleagues in the Center for Cancer Systems Biology at Stanford integrated the gene expression patterns of 39 types of cancer from nearly 18,000 cases with information on how long each patient survived. Their analysis of the resulting database, PRECOG, identified small sets of genes that are associated across a surprisingly broad spectrum of cancer types—with either good or bad patient prognoses. They then applied Cibersort to the cases they'd analyzed, discerning complex associations between patient survival and the presence of some 22 distinct types of immune cells in tumors.

An eye on patients

Aside from pipettes, food and reagents, Alizadeh and Diehn also share a guiding principle. "We both try to integrate our lab work and clinical work as much as possible," says Diehn. Alizadeh, a clinical oncologist, specializes in lymphoma, while Diehn, a radiation oncologist, focuses on lung cancer. These are, respectively, among the most common blood cancer and solid-tumor types. "We think what we're working on in these areas should extend to cancer broadly, in line with Ludwig's mission," says Alizadeh. "So we try to take moonshot types of experiments and reduce them to practice, to what can be done for the average patient."

Consider their work with circulating tumor DNA (ctDNA), which is shed by dead cancer cells and can, with some analytical finesse and the latest technologies, be detected in blood and other fluids. "We both became interested in ctDNA as part of our clinical routine," says Diehn. "Ash was running some assays available for lymphoma patients, and I was frustrated that there are no good biochemical markers for lung cancer."

In 2014, the pair reported in *Nature Medicine* a highly sensitive, minimally invasive method for detecting non-small cell lung cancers (NSCLCs) in patients. Their technique, CAPP-Seq, detected 50% of Stage I NSCLC, and 100% of Stage II-IV NSCLCs. The researchers also found that they could assess treatment responses earlier than they did using radiologic imaging. They are now developing more sensitive tests for Stage I NSCLC–when the cancer is most treatable–and similar tests for other malignancies, including those of the blood, brain and gastrointestinal tract.

These applications were enabled by improvements the researchers made to CAPP-Seq. Their new method, named integrated digital error suppression (iDES), combines two clever and complementary strategies to eliminate errors introduced when ctDNA is captured and prepared for sequencing and was reported in a March 2016 paper in *Nature Biotechnology*.

"Could we use it for screening? That, of course, is the Holy Grail and the hardest problem," says Diehn. "But we could also use it to monitor response to drugs or the



development of drug resistance in patients. Those are just some of the projects we're working on."

Hitting rewind

Cibersort likewise had its roots in a clinical problem.

The severity of a cancer is often tied to the diversity of cells in a tumor, and information on that diversity can be key to effective treatment. Pathologists and researchers get a handle on that diversity today through flow cytometry, in which cells in a sample are separated and then labeled and counted using antibodies, or via microscopy. This is relatively easy when dealing with blood. But tearing solid tumors apart can destroy certain types of cells and, in either case, the antibodies required for a comprehensive profile are not always available.



Ash Alizadeh Ludwig Stanford

"When Aaron Newman, a very talented postdoc in our group, first approached me about trying to tackle this problem computationally, I was skeptical," recalls Alizadeh. Newman was proposing to turn tissue into the equivalent of a smoothie and use the molecular clues in there, plus some software, to identify the cell types in the intact tissue. Others had tried similar approaches before with uneven results. "But he took up the challenge with such fierce and unwavering commitment that he produced something pretty powerful," says Alizadeh.

One big challenge in any such analysis is that all cells express a basic subset of housekeeping genes, making for a lot of data "noise" through which the unique signal of a specific type of cell must be detected. Another is that any tissue sample is likely to contain cell types the computer does not know about and so cannot take into account in its analysis.

To build Cibersort, the researchers read the transcripts of expressed genes from about 20 cell types and developed a fingerprint for each based on about 500 or so genes each characteristically expresses. Newman then applied machine-learning algorithms—the sort of programs used in speech recognition and self-driving cars to address the anticipated data noise and confusion, and to reconstruct the tissue based on those fingerprints.

Cibersort requires much less work and introduces fewer variables than current methods for analyzing cell types in tumors, says Diehn. It is also sublimely precise. "We've been able to detect very closely



Maximilian Diehn Ludwig Stanford

related yet distinct subsets of immune cells, down to really low fractions of one percent or less of the total," he notes. "We can even discern active subsets of immune cells from inactive ones."

Good prognoses and bad

PRECOG too addresses a sticky problem in cancer research. Researchers have not been able to figure out precisely how the profiles of genes expressed in tumors correspond to outcomes in most cancers. Many have found patterns, but such findings have largely been hard to replicate, except in a few types of malignancies like breast cancer. This, says Alizadeh, is because most such studies were too small compared with the number of genes expressed by cancer cells.

But about five years ago, the researchers noticed that there was a critical mass of relevant data available: Tumor samples from tens of thousands of patients had been genetically profiled and stored along with their clinical outcomes.

Working with their colleagues at the Stanford Center for Cancer Systems Biology, including Andrew Gentles and Sylvia Plevritis, the researchers built a database that they named PRECOG by curating data from their own studies and those of their past collaborators, and by browsing information deposited in public repositories. The results of their analysis were surprising.

"Across the 39 cancer types we looked at, tumors had far more in common than they did distinguishing features when it came to patient prognosis," says Alizadeh. "About two-thirds of the genes that are prognostic for one cancer are prognostic for at least one other cancer type."

The team was able to identify the top 10 genes broadly associated with each prognosis. In particular, high expression of FOXM1, a gene involved in cell growth, was associated with a poor prognosis across cancers. Meanwhile, the expression of a gene known to be involved in immune responses, KLRB1, seemed to have as broad a protective effect.

To get a better sense of the immune component of outcomes, the researchers applied Cibersort to the problem, painting a sweeping portrait of the association of immune cells with prognoses.

"Immune cell contributions can be upsidedown from cancer to cancer," says Alizadeh. "An immune cell like a macrophage can be favorably prognostic in a lymphoma, but very adversely so in, say, a breast cancer. If you were trying to engage macrophages with an immunotherapy, you might want to know about that."

PRECOG, which is freely accessible, has obvious implications for cancer research and the development of new therapies and diagnostics. Diehn has already led a study, published in the *Journal of the National Cancer Institute* in 2015, reporting a potential diagnostic test for NSCLC based in part on information gleaned from PRECOG.

"The test predicts which patients will benefit from more aggressive, systemic therapy after having lung tumors removed in early stage lung cancers," says Diehn. The test would have to be validated in a large clinical trial, he notes, but because it only requires detection of nine genes, most clinical labs would be able to perform the assessment.

"As oncologists," says Alizadeh, "we are often humbled by the fact that we're shooting in the dark and lack the tools we need to see the responses we're hoping to see. But we're very hopeful."

With good reason, it would appear. L

The researchers built the PRECOG database by curating data from their own studies and past collaborations, and with information in public repositories. The results were surprising.

"One of the key questions we're pursuing is how the rearrangement of the epigenetic landscape occurs in cancer."

SKIRMANTAS KRIAUCIONIS

NUCLEAR SABOTEUR

Skirmantas Kriaucionis was a postdoctoral researcher in Nathaniel Heintz's laboratory at Rockefeller University when he noticed, in 2008, an inexplicable but recurrent blip in his data from experiments with mouse brain cells. He and Heintz could have ignored it and carried on with the work at hand. But something told them to look a little deeper. This something was right. After much research into the blip, they concluded it harbored that rarest of scientific gems: a fundamental biological discovery. Their findings, reported in *Science* in 2009, added a new DNA base—5hydroxymethylcytosine (5hmC), previously seen only in bacteria to the known chemical alphabet of the mammalian genome.

A similar mix of serendipity, insight and persistence paid off for Kriaucionis in 2015. As head of his own lab today at Ludwig Oxford, Kriaucionis focuses on how chemical, or "epigenetic," tags added to DNA—like 5hmC and its classical counterpart, 5-methylcytosine (5mC) alter gene expression. Such tags help determine which genes are expressed by a given cell, giving each type its specialized function and explaining how a single genome can lead to things as disparate as a taste-bud and a liver.

Epigenetic marks of all sorts have also long been known to be rampantly misplaced across the genomes of cancer cells. Kriaucionis and his team were testing a theory of how this might occur when they made a discovery that had unexpected implications. Their findings, published in *Nature* in 2015, show that a characteristic sloppiness in the way some types of cancer cells handle epigenetically marked bases may be harnessed to devise a new kind of therapy for various cancers.

From pond to lab

Kriaucionis grew up in Kaunas, a city at the confluence of Lithuania's two largest rivers. His mother worked as a bookkeeper and his father as a driver for the local university. His parents often took him to the country, where the family owned a small plot of land and the young Kriaucionis could indulge his interest in bugs and pond life. His parents, he recalls, actively nurtured his budding interest in science, buying him magnifying glasses and microscopes along with the classics of his favorite science fiction author Jules Verne.

"I was fascinated by how the living world works," says Kriaucionis. "My parents weren't scientists but they were very attentive to their children. That, I think, made the biggest difference."

Naturally, Kriaucionis majored in biology when he enrolled in Vytautas Magnus University in Lithuania's capital Vilnius. There, he was introduced to epigenetics during his thesis research with Saulius Klimasauskas, an authority on the phenomenon in bacteria. He was hooked. For his doctoral research with Adrian Bird at the Wellcome Trust Centre for Cell Biology at the University of Edinburgh, Kriaucionis studied how proteins recognize 5mC, which cells employ to switch genes off.

The researchers found that some types of cancer cells tweak modified nucleosides, permitting their incorporation into new DNA. The practice, however, often kills the cells. After a stint as a postdoc in Bird's lab, Kriaucionis landed a second fellowship in Heintz's lab at Rockefeller University, where he began exploring why the nuclei of two types of brain cells appeared so different in their organization. That was the research that yielded the discovery of 5hmC which, unlike 5mc, seems highly enriched in brain cells and in stem cells during early development. It too is broadly misplaced across cancer genomes.

Useful error

Since joining Ludwig Oxford in 2010, Kriaucionis has continued to probe the epigenetics of normal and cancer cell biology. In 2012, he and Heintz led a study published in *Cell* showing that 5hmC is associated with genes that are actively expressed and that its presence is detected by the same protein, MeCP2, that recognizes 5mC.

These findings were of relevance to Rett syndrome, a developmental disorder that varies in severity depending on how precisely MeCP2 has been altered. Heintz, Kriaucionis and their colleagues reported that a mutant MeCP2 protein associated with less severe cognitive and speech deficits in Rett patients is capable of binding 5mC, but not 5hmC.

Since then, Kriaucionis has dug deeper into the biology of 5mC and 5hmC. "One of the key questions we're pursuing is how the rearrangement of the epigenetic landscape occurs in cancer," says Kriaucionis. There are two possibilities. One is that the aberrant signaling within the cancer cell induces the effect. The other is that the process is random, and particular epigenetic patterns are ultimately favored because they promote the survival of the cells that harbor them.

As part of their exploration of the latter possibility, Kriaucionis and his colleagues

examined whether modified bases are randomly incorporated into the genomes of cancerous cells. Such bases might come from last night's steak, or from the body's own stew of metabolic byproducts.

The researchers found that the enzymes that help recycle DNA bases—which are borne by molecules known as nucleosides are highly specific. They reject modified bases, ensuring that the new DNA is epigenetically "clean." When the researchers looked at the recycling process in cancer cell lines, they discovered that some types of cancer cells tend to chemically tweak modified nucleosides picked up from the recycling pool, permitting their incorporation into new DNA. The practice, however, often kills the cells.

They showed, critically, that cancer cells that express unusually high levels of a protein called cytidine deaminase (CDA) are prone to such errors. Previous studies have shown that a number of cancers from those of the pancreas to the stomach to the testes—overexpress this enzyme. But the phenomenon had been seen as a means by which tumors resist chemotherapies like gemcitabine, which are essentially modified nucleosides designed to kill rapidly dividing cells.

Kriaucionis and his team realized, however, that their modified nucleosides, including 5hmC, were likely to have the opposite effect. Better still, they showed this to be true, at least in an animal model. "The modified nucleosides we used actually kill cells that over-express CDA," says Kriaucionis.

The researchers are now beginning studies to determine whether their nucleosides are amenable to translation into viable candidate drugs for evaluation in human studies. "We are especially keen



Skirmantas Kriaucionis Ludwig Oxford

to determine whether these compounds work against pancreatic cancer," says Kriaucionis. "It is a very aggressive malignancy that overexpresses CDA and is highly resistant to treatment. Current therapy does very poorly for patients. It would be very rewarding if we could improve their outcomes."

MOVING DISCOVERIES





Mutations that hit coding genes can result in the production of aberrant proteins.

1000

LUIS DIAZ

TUMOR TARGETER

About four years ago, Luis Diaz walked into Bert Vogelstein's office at Ludwig Johns Hopkins and announced that he'd just had something of a scientific insight. Diaz, an oncologist and accomplished cancer geneticist, had been watching the progress of a class of cancer immunotherapies known as checkpoint blockade with a touch of surprise. As the son of a prominent immunologist, he had grown up virtually breathing immunology and had an instinctual feel for the subject. "I often say that immunology is my hobby," he says. "But I'd always believed it would be very tough to elicit an immune response against a tumor. In fact, until relatively recently, I didn't think we'd ever have an immunotherapeutic approach that would work."

Now, Diaz told Vogelstein, co-director of Ludwig Johns Hopkins, he thought he knew why antibodies against a protein named programmed death-1 (PD-1) were eliciting intense anti-tumor immune responses in some patients. The cancer cells in responsive patients, Diaz suspected, were laden with many more mutations across their genomes than those of patients who had not responded to the therapy. This suggested, he said, that cancers of any type that are deficient in their ability to repair DNA might be susceptible to checkpoint blockade.

His hunch laid the foundation for a clinical trial whose results—reported at the 2015 American Society for Clinical Oncology Annual Meeting and published in the New England Journal of Medicine (NEJM)—thrilled the oncology community. Diaz and his colleagues found that, regardless of their tissues of origin, tumors whose cells are deficient in repairing mismatched DNA sequences, and so preventing a gross accumulation of mutations, are far more susceptible to the anti-PD-1 antibody pembrolizumab than those that retain this ability. Equally important, candidates for such treatment can be easily identified by genetic tests that have been on the market for about two decades.

From hallway to clinic

Diaz's hypothesis may have been a mite premature back in 2012, but he and

Vogelstein nonetheless shot off a letter on the matter to NEJM. The journal promptly rejected their proposal.

Still, Diaz believed he was onto something, and he had found an enthusiastic sounding board for his ideas. Vogelstein—and, independently, Ludwig San Diego Director Richard Kolodner—had in the early 1990s discovered the genetic basis of an inherited propensity for colon cancer known as Lynch syndrome. They had shown that Lynch patients had defects in genes that repair DNA, making them prone to mutations of all sorts, including those that cause cancer.

Diaz, who specializes in treating colon cancer, also knew that the tumors of Lynch patients tended to be highly infiltrated with immune cells and that these patients live longer with their cancers than do most other colon cancer patients. Meanwhile, clinical studies were showing that melanomas respond quite well to PD-1 blockade. These tumors, like those of tobacco-related lung cancers, are known to have highly mutated cells.

BETTER TOGETHER Jedd Wolchok and Stephen Hodi

The evaluation of mechanistically distinct immunotherapies in combination for a variety of cancer types is among the most intriguing trends in cancer research. Jedd Wolchok of Ludwig MSK and Stephen Hodi of Ludwig Harvard are among the pioneers of the strategy, testing the effects of combination checkpoint blockade in patients with advanced melanoma. In 2015, they caused a bit of a stir in the medical community with their publication of the results of a multicenter, Phase 3 trial they led.

The study, which was funded by Bristol-Myers Squibb showed that a combination of the CTLA-4 inhibitor ipilimumab and PD-1 inhibitor nivolumab induces more frequent responses and considerably longer progression-free survival in patients with advanced melanoma than the administration of either of them alone. Published in the *New England Journal of Medicine*, these results prompted the US Food and Drug Administration to approve the combination for patients with advanced, inoperable melanoma.

Wolchok, Hodi and their colleagues found that for ipilimumab alone, the median overall progression-free survival (PFS)—the length of time following treatment before the cancer resumes its growth—was 2.9 months. Patients treated with nivolumab alone had a median PFS of 6.9 months, while the combination of the two resulted in a PFS of 11.5 months. The team also reported that 19% of patients treated with ipilimumab alone and 44% treated with nivolumab had an objective response to each therapy, measured as a significant reduction in tumor size. The response rate for the combination therapy was 58%.

CTLA-4 is a protein found on T cells, which can destroy cancerous and diseased cells. When switched on, it tamps down T cell activity. PD-1, also found on the surface of T cells, He and Vogelstein began discussing the idea with colleagues at Johns Hopkins. They learned in those discussions that anti-PD1 antibodies had generally failed to induce responses in one trial involving colon cancer patients. But, in a casual hallway conversation, Diaz learned that one patient out of the 33 enrolled in that trial had in fact responded rather well. Diaz asked that the tumor sample from that patient be tested for its mutational load.

"Colon cancer cells typically only have a few dozen mutations," says Diaz. "But we

were thinking, maybe that patient's tumors had mismatch repair deficiencies and would harbor thousands of mutations per cell. And, lo and behold, that turned out to be the case."

Excited, Diaz and Vogelstein asked Merckwhich makes pembrolizumab-and other companies making anti-PD-1 antibodies whether they would be interested in supporting a trial testing his idea. The answer was, uniformly, no. Coaxed and cajoled by Diaz, however, Merck finally gave in a little: it would donate the drug, but Diaz would

is activated by a protein known as PD-L1, an event that prompts T cells to selfdestruct. Both proteins prevent excessive autoimmunity and the destruction of healthy tissues following immune responses to infections. Many tumor cells, however, hijack this protective mechanism and express PD-L1 to thwart T cell attack.

Since its FDA approval, the combination therapy has been welcomed by oncologists, who need every edge they can get against this remarkably aggressive malignancy.

"It has definitely caught on," says Wolchok. "Here at MSK it's our go-to option for people who we feel have the medical reserve for some of the side effects that may occur with the treatment." Those side effects, he points out, can be managed in many patients.

Wolchok, Hodi and their colleagues continue to collect data on the overall survival of patients who participated in the Phase 3 trial.



have to find the funding elsewhere and agree to sponsor the trial—accepting liability and responsibility for its management.

"Fortunately," says Diaz, "we got support for the trial from the philanthropy Swim Across America, which, along with Ludwig, supports my research. We were able to run the trial on a shoestring budget."

Green lights

Diaz recruited a young gastrointestinal oncologist, Dung Le, an assistant professor of oncology at Johns Hopkins, to lead the study with him. Their clinical trial involved three cohorts from a total of 41 patients, all of whom had very advanced cancers. One included patients with colon cancer that was deficient in DNA repair. The second enrolled patients with a variety of other cancers that were similarly dysfunctional, while the third included colon cancer patients whose tumors were proficient in such repair. All patients were given pembrolizumab, after which they were evaluated for reduction in

"Right now our focus is on colon cancer," says Diaz, "but I can tell you that this is probably going to be tumor-type independent, as this genetic marker is found across a variety of cancers."



tumor size (immune-related objective response rate, or irORR) and for progression of disease at 20 weeks (progression-free survival, or irPFS).

The results were stunning. The DNA repairdeficient colon cancer patients, many of whom were at death's door when they entered the trial, had an irORR of 40% and an irPFS of 78%. Patients with other DNA repair-deficient cancers had an irORR of 71% and an irPFS of 67%. None of the colon cancer patients whose tumor cells could repair DNA responded to the therapy, and this cohort's irPFS at 20 weeks was only 18%. Diaz and his colleagues reported that DNA repair-deficient tumors harbor more than 20 times as many mutations as proficient ones. High rates of mutation, they found, are associated with prolonged progression-free survival following PD-1 blockade.



Luis Diaz Ludwig Johns Hopkins

That makes sense. Mutations that hit coding genes can result in the production of aberrant proteins. These may be seen by the immune system as foreign, prompting a response lethal to cancer cells. It is this response that would be further stimulated by checkpoint blockade.

Merck was excited by the results: It immediately launched two large scale trials led by Diaz and Le to obtain regulatory approval for the therapy, one of them as first-line therapy for DNA repair-deficient colon cancers. The US Food and Drug Administration was impressed as well. It gave the therapy "breakthrough" status in November to speed its path to the clinic.

"Right now our focus is on colon cancer," says Diaz, "but I can tell you that this is probably going to be tumor-type independent, as this genetic marker is found across a variety of cancers. We think the eligible patients may represent as many as one in 25 of all cancers."

Diaz, for his part, is most excited for his patients.

"I would walk into the room of a man who was being consented for hospice, give him a drug and watch his tumor melt away," says Diaz, recalling the thrill of the trial. "These patients typically had just weeks to live when they enrolled. More than half of them had a major response to the therapy. Some had complete responses. It's still very satisfying to continually interact with people who would not be living today if they hadn't been offered this therapy."

"What we've all learned is that there's a wide gulf between identifying a drug target and having a drug actually work."

PAUL MISCHEL

CANCER'S CIRCUIT BREAKER

In 1938, a 13-year-old Theodore Mischel was, along with the rest of his family, frantically destroying all evidence of their Jewish heritage when his eight-year-old brother found a document showing their maternal grandfather had at some point become an American citizen. It sufficed to get them passage to the U.S. as refugees just after German forces swept into Austria to establish the *Anschluss*. Five years later, Theodore had enlisted in the US military and was *en route* to what would come to be known as the Battle of the Bulge when he came down with the mumps.

He was confined to a field hospital and, by the time he recovered, the tide had turned in favor of the Allies. So he was assigned to the intelligence corps, with which he served as a translator during the Dachau concentration camp trials. Having possibly dodged death twice before turning 20, Theodore went to college on the GI Bill, eventually becoming a professor of philosophy in upstate New York.

"He's living the American dream," recalls his son Paul Mischel, who is today a member of Ludwig San Diego, "and then, at 51, he gets diagnosed with stomach cancer. He dies in this absolutely excruciating fashion. I was 14, and it was heart-wrenching listening to people say, 'Well, at least we caught it early'. Of course, it's rarely caught early. I watched him become a human skeleton within six months and decided then that I would dedicate myself to doing something about this disease."

Mischel has picked as tough a quarry as you get in pursuit of that goal. He focuses on glioblastoma multiforme (GBM), an incurable brain cancer that typically takes the lives of patients within 15 months of their diagnosis. Working with his colleagues—most notably Web Cavenee, who today directs Ludwig's alliances in brain cancers, (see Box, page 37) and Frank Furnari of Ludwig San Diego— Mischel has over the past decade explored how the GBM cell's genome, metabolism and responses to the environment interact to support tumor growth and drug resistance. Working with the laboratory of his Ludwig San Diego colleague Bing Ren (see story, page 9) in 2015, Mischel and his team charted in granular detail how an aberrantly activated mutant receptor alters the chemical, or "epigenetic," modification and reading of the GBM genome through a protein complex known to coordinate cancer cell metabolism. He also led a study that showed how two common nutrients, glucose and acetate, can drive drug resistance through that same complex, known as mTORC2. Both studies have clinical implications. The former unveiled a promising therapeutic strategy for GBM. The latter not only revealed a novel mechanism of cancer drug resistance but also exposed the potentially counterproductive effects of a drug often given to GBM patients.

Tracing circuits

Mischel went to medical school at Cornell University and then trained as a cancer pathologist before taking a fellowship in molecular neurobiology at the University of California, San Francisco. After joining the faculty of UCLA in 1998, he continued his studies charting the biochemical cascades responsible for signaling within cells. When distorted, such signals drive the uncontrolled growth of cancer cells, and the proteins responsible for transmitting them are the targets of many modern cancer drugs.

Though such targeted therapies have certainly improved outcomes for some cancers, they've been far less successful than was initially expected. GBM has, at any rate, shrugged off every targeted therapy thrown at it by researchers.

"What we've all learned," says Mischel, "is that there's a wide gulf between identifying a drug target and having a drug actually work."

Mischel wants to know why. Since moving to Ludwig in 2012, he and his longtime

collaborators have uncovered seemingly inexhaustible mechanisms by which GBM cells adapt to those few therapies that actually make it into the tumor. They've found that GBM cells switch signaling circuits when a preferred pathway is blocked by a drug, that they change the cell surface receptors—think of them as the switches that engage those circuits. Most bafflingly, they even found that GBM cells can "hide" the mutant genes that encode an aberrant receptor, EGF receptor vIII (EGFRvIII), until an EGFR-targeting therapy is halted.

Looking deeper

Such findings have inspired Mischel to look at the cancer cell and its genetic programs in a new way.

"We have had a mechanistic view of cancer genes," says Mischel. "We put them into models and see that they replicate tumors, but we don't really understand how they change the cell or what they do that causes cancer."

One place in which he is looking for that perspective is in the induction of the cancer cell's uniquely productive metabolism. Mischel and other researchers have shown that the protein complex mTORC2 is a central controller of the phenomenon. Its activation by such drivers of cancer as EGFRvIII cranks up, among other things, the import of glucose and acetate. These nutrients provide raw energy to cells and, through a metabolic sleight of hand known as the Warburg effect, furnish the molecular building blocks required to make new cells.

In one study, published in the Proceedings of the National Academy of Sciences in 2015, Mischel and his colleagues showed that in GBM cells driven by EGFRvIII, the boost in glucose and acetate uptake through mTORC2 activation has an additional effect: It induces drug resistance. They report that a



NECESSARILY AGILE

Web Cavenee

Web Cavenee has over the past three decades contributed immensely to our understanding of the molecular drivers of glioblastoma multiforme (GBM), an aggressive and highly adaptive brain cancer. His characterization of a mutant epidermal growth factor receptor-EGFRvIII-and its role in GBM have put Ludwig's San Diego Branch on the map as a leading center of brain cancer research. Cavenee also helped lead a team of Ludwig researchers that developed a uniquely targeted antibody against EGFRvIII, which is the mutant form of the receptor most frequently found in GBM tumors. The drug company AbbVie has since "armed" the antibody with a toxin to turn it into a guided missile against GBM cells and taken it into clinical trials.

In 2015, Cavenee handed over the reins of Ludwig San Diego to its new director, the equally accomplished cancer geneticist Richard Kolodner, and took on a new role as Ludwig's director of strategic alliances in central nervous system cancers. He has been as busy as ever in this new role, joining a global team of researchers last year to announce the launch of a new kind of clinical trial to find effective therapies for GBM.

Named GBM AGILE, it will involve more than 130 clinical and laboratory researchers from the US, China, Australia and Europe. Unlike typical clinical trials, GBM AGILE is devised to permit researchers to not only tailor their treatments to the molecular profiles of GBM tumors but also to drop failed treatment strategies in midstream and apply new ones as new information about the cancer and its treatment comes to light. This applies to the various arms of the trial itself as well as to individual patients.

Cavenee will help lead GBM AGILE with Anna Barker of Arizona State University and Al Yung of MD Anderson Cancer Center. The trial, which is expected to begin enrolling patients by mid-2016, will apply Bayesian statistics to interpret the data it collects. Its primary aims are to test more individualized combination therapies for GBM and to begin validating novel biomarkers to guide such treatment an effort that will be led by Ludwig San Diego's Paul Mischel (see story above). "If we could target c-Myc, or some of the players along the way that regulate c-Myc... we might actually be able to make a real difference for patients."

shared metabolite of the two nutrients, acetyl-co-A, directly activates mTORC2 in cells treated with a targeted therapy against EGFRvIII. This effectively circumvents the blockade on signaling that the drug is meant to impose—and illustrates the ability of the cancer cell to adapt to its environment (the threat of a drug) in a manner that is not directly dependent on genetic change.

The finding is also of immediate clinical relevance. GBM patients are often treated with steroids to contain brain inflammation, and steroids tend to ramp up blood glucose levels. Such therapy, it seems, may inadvertently fuel the growth of GBM tumors.

A peek at the sourcecode

In a second study, published in *Molecular Cell*, Mischel partnered with Ludwig San Diego's Ren to examine how exactly EGFRvIII alters the reading of the GBM genome. Using technology developed in Ren's laboratory (see story, page 9), the researchers began by profiling EGFRvIII's epigenetic activation of DNA sequences known as "enhancers." These elements of DNA do not themselves encode anything. Instead, they boost the expression of specific genes.

Most of the enhancers they identified bore signature DNA sequences that are bound by dozens of transcription factors—regulators of gene expression—expressed at high levels in GBM. Two of the signatures stood out: those for the transcription factors SOX9 and FOXG1. Notably, their silencing in experiments stopped tumor growth, both in cell cultures and in an animal model that mimics GBM.

The researchers next examined the genes whose expression is controlled by SOX9 and FOXG1. One of those genes turns out to be a protein named BRD4, which in turn is known to control the expression of another transcription factor named c-Myc, a molecular lever that links signals driving growth to those that control metabolism. Working with Cavenee and Furnari, Mischel has uncovered several distinct mechanisms by which mTORC2 induces the aberrant activation of c-Myc in GBM.

"Our studies are converging to show how EGFRvIII is reprogramming the metabolism in GBM cells through c-Myc," says Mischel. "This suggests that if we could target c-Myc, or some of the players along the way that regulate c-Myc, like BRD4, we might actually be able to make a real difference for patients."

To test that hunch, the researchers tapped the expertise of the Ludwig Cancer Research Small Molecule Discovery Program, headed by Andrew Shiau (see story, page 13). Together, they showed that an experimental drug named JQ1, which is currently in clinical trials for another cancer, could kill EGFRvIIIfueled GBM cells and shrink tumors in a mouse model.

Mischel and his colleagues are digging deeper into how the epigenetic changes they've



Paul Mischel Ludwig San Diego

mapped drive GBM. They're also working on developing novel molecules to target c-Myc activation as possible drug candidates.

"We're actively asking how changes in the environment change the levels, the activities and the consequences of cancer genes," says Mischel. "We hope and expect that this work will connect to some intelligently designed clinical trials and, perhaps, bring new hope to patients diagnosed with this cancer."

Ludwig and CRI have for many years been at the leading edge of cancer immunology and immunotherapy

CANCER RESEARCH INSTITUTE

A MARRIAGE OF LIKE MINDS

At some point in the early 1990s, Ludwig's former CEO and scientific director Lloyd Old concluded that the field of tumor immunology had matured sufficiently to have its implications put to the test in clinical trials. A legendary cancer immunologist, Old had helped launch the Ludwig Institute for Cancer Research in 1971, right around the time he was appointed medical director of the Cancer Research Institute (CRI). Over the next quarter century, he worked with researchers in both organizations to help build the scientific foundations of cancer immunotherapy.

Under Old's direction, Ludwig and CRI had by the mid-1990s both begun funding small immunotherapy trials. "After a few years of doing this, we looked back and realized we really hadn't moved the dime," recalls Jill O'Donnell-Tormey, who is today CEO and scientific director of CRI. The problem, Old concluded, was that there was too little coordination between the funded researchers and their various studies. A more cohesive effort was in order. That recognition culminated in the establishment of a research network that Old named the Cancer Vaccine Collaborative. The network's scientists, based mainly in New York at the outset, were tasked with developing an effective cancer vaccine.

Fifteen years on, that venture has expanded to include leading clinical and research immunologists in a dozen countries on four continents and is now known as the CVC Clinical Trials Network, or CVC for short. It has forged partnerships with 15 pharma and biotechnology companies and become a vital force in the design and testing of novel immunotherapeutic concepts and combination strategies. In 2015, Ludwig and CRI launched two new immunotherapy trials through the CVC, bringing the total running under its banner to five. One is testing the effects of durvalumab, a checkpoint blockade antibody against PD-L1 made by MedImmune, as a treatment for the aggressive brain cancer glioblastoma multiforme (GBM). The other, led by George Coukos, director

of Ludwig Lausanne, seeks to treat advanced, drug-resistant ovarian cancer by combining durvalumab with another investigational immunotherapy named Motolimod—a Tolllike receptor 8 (TLR-8) agonist—made by VentiRx Pharmaceuticals.

"Ludwig and CRI have for many years been at the leading edge of cancer immunology and immunotherapy," says Jonathan Skipper, Ludwig's executive vice president for technology development. "We've supported many studies critical to the advancement of this promising therapeutic strategy and we plan to maintain our leadership in the field."

The New York Protein

By 2001, Ludwig had already built a formidable infrastructure for translational research. It was also assembling a capable clinical trials management team that could support global, multicenter studies and had rights to NY-ESO-1, a protein Old had codiscovered that is found almost exclusively on cancer cells. Preclinical studies suggested it showed promise as a target antigen for a cancer vaccine.

The fledgling CVC began studying how best to design, formulate and deliver an NY-ESO-1 vaccine. "It took about 10 years and over 50 small trials getting there," says O'Donnell-Tormey. "Our candidate vaccine elicited potent anti-vaccine immune responses, but we couldn't consistently see clinical responses in patients."

The effort wasn't wasted, however. Ludwig's Chief Medical Officer Ralph Venhaus points out that a decade of honing and testing the NY-ESO-1 vaccine turned the members of Ludwig's clinical management team into experts in immunotherapy trials. The studies had been equally instructive for the growing network of CVC immunologists. As a bonus, they now had a viable cancer vaccine candidate, one that elicited the right kinds of immune responses. Old, who died in 2011 of prostate cancer, suspected it just needed some kind of extra immunotherapeutic boost to cut past the tumors' defenses.

Catching a wave

Such candidate therapies, as it turned out, were coming up fast in the industrial pipeline, and the CVC's clinical immunologists wanted to get their hands on them. Some in earlier stages of development (like GITR agonists, which Ludwig and CRI are developing) were designed to directly amp up anti-tumor immune responses. Others-particularly the checkpoint blockade antibodies against cell surface proteins PD-1 and CTLA-4released the brakes the immune system imposes on its cellular foot-soldiers. These, pushed by Ludwig MSK's former director James Allison and others, were closer to market, or already there by 2011. But the network's researchers couldn't get their hands on any of them.

The trouble was drug companies were not enthusiastic about academic researchers interfering with the development of their products and even less so about supporting the expense and administrative bandwidth required to run the trials that the scientists requested. There were strategic barriers as well, especially when it came to combination therapies-a particular goal of Ludwig and CRI-since agents of interest were frequently owned by different companies. "Back then," says Skipper, "different companies very rarely tested their products together in clinical trials, let alone their investigational agents." Yet the network's true potential could only be unleashed if its immunologists had access to proprietary, investigational agents.

Aware of this, Adam Kolom—a former private equity investor who had devised and brought to CRI a philanthropic venture capital mechanism to hasten drug development began working with Skipper to identify ways to overcome industry's resistance. Their model powers the network today. "It removes the obstacles to getting proprietary agents into the hands of the CVC's academic researchers, allowing them to do their most ambitious clinical research," says Kolom, who is managing director of the CRI Venture Fund. "We're like a Make-A-Wish Foundation for our principal investigators."

The wishing machine

The model is structured to be guided above all by the needs of Ludwig and CRI's primary constituencies: cancer patients and clinical researchers. Patients who enroll in the trials get early access to cutting edge combination therapies or to agents that might not otherwise have been used for their particular type of cancer. The CVC trial examining checkpoint blockade for GBM—a swiftly lethal cancer for which there are essentially no effective treatments—falls into the latter category.

The researchers, meanwhile, get to ask important clinical and scientific questions using rigorously characterized and clinically pedigreed samples obtained from trial participants. They also set the research agenda and get hassle-free access to the proprietary drugs they need to test their clinical hypotheses. "We come to the researchers with a virtually turnkey operation," says O'Donnell-Tormey. CRI calls the combination of all these elements—the venture fund, access to experimental drugs, the partnership with Ludwig—the Clinical Accelerator.

The CVC's management team includes, among others, Skipper, Kolom, O'Donnell-Tormey, Venhaus, Vanessa Lucey, associate director of the Clinical Accelerator at CRI, and Ludwig MSK's Jedd Wolchok, who



Jonathan C.A. Skipper Executive Vice President for Technology Development

serves as director of the CVC Clinical Trials Network. The team canvasses the opinions of network members about the key questions that the field would like answered. "In no way is this a monarchy," says Wolchok, "nor is it an anarchy. This is crowdsourcing at the highest level."

Once the leadership team has picked winning hypotheses, Skipper, Kolom and their colleagues scan the industry for agents essential to their evaluation. If, as is often the case, drugs from different companies are to be tested together, they have developed a formula for managing data access, safety reporting, publication rights and intellectual property that makes the partnership as painless as possible for all parties.

Such collaborations are of obvious benefit to small, possibly cash-strapped biotech startups. But they're also of value to larger pharma players. "We're effectively doing a business development function for them," notes Skipper. "If the combination therapy tested in a trial proves successful, they will not only have clinical data supporting the new immunotherapeutic



Ralph Venhaus Vice President for Clinical Trials Management

strategy for their drug, but will have also found a partner for product development without having had to invest very much in the usual due diligence."

The drug companies also benefit from access to the CVC's brain-trust of leading cancer immunologists. "We hear from MedImmune and our other pharma partners that this opportunity to interact with people who have a depth of experience in immune oncology is very valuable to them," says Wolchok. "The occupational half-life of someone in academia is quite a bit longer than that of an equally qualified person in industry, so it's good for them to have a stable source of cognitive and clinical power."

A gazillion little wheels

To alleviate the financial concerns of companies, CRI covers a share of the cost of running clinical trials through its non-profit CRI Venture Fund, which is structured to be replenished by successbased milestone payments from partner firms. Another obstacle, industry's reluctance to take on the sponsorship and management of externally proposed trials is solved by Ludwig's Clinical Trial Management (CTM) team, which is well versed in the requirements of regulatory agencies.

"The Clinical Trials Management operation has a gazillion little wheels," says Venhaus. "It is a giant project."

Ludwig's CTM oversees everything from creating trial protocols that meet industry standards to obtaining approvals from ethics boards and regulatory agencies. It also vets and prepares clinical trial sites and oversees the conduct of the trials. The CTM, further, manages the proper collection, processing and storage of the clinical samples—a resource that has yielded critical insights into the molecular and cellular biology of immunotherapeutic responses.

The CTM's institutional experience with immunotherapy has proved invaluable. "Because of that knowhow, we can take an idea on the back of an envelope and turn it into a workable, fully developed clinical trial protocol," says Venhaus. "If researchers wanted to get that done with a contract research organization, they'd have to spend weeks and weeks to get them to execute it correctly."

Online and active

In 2012, Ludwig and CRI launched an ongoing partnership with MedImmune, the global biologics research and development arm of AstraZeneca. The agreement gave CVC researchers access to the company's checkpoint antibody portfolio for evaluation alone or in combination with other immunotherapies.

Such combinations are a particular focus of the field, thanks in substantial measure to work done by Wolchok and other researchers demonstrating their complementary effects in advanced melanoma. Wolchok is co-chair of a CVC trial running now in which durvalumab and MedImmune's anti-CTLA-4 antibody, tremelimumab, are being used together to treat a variety of other solid tumors. Many patients in the trial might not otherwise have had access to these promising agents as treatments for their particular malignancies.

At the same time, Ludwig and CRI are increasingly turning their attention to targeting less exploited immunologic pathways to target tumors. They recently signed, for example, an agreement with the biotech Targovax to test its candidate oncolytic virotherapy-in which an engineered virus is used to target tumors-with other immunotherapies. This trial too is based on a preclinical study in which Wolchok and Ludwig MSK's Dmitriy Zamarin showed that a separate oncolytic virus they're developing induced dramatic regressions of tumors in a mouse model of melanoma when it was delivered with CTLA-4 blockade.

The ovarian cancer trial being led by Ludwig Lausanne's Coukos is another such example. It combines durvalumab with VentiRx's drug Motolimod to treat drug-resistant ovarian cancers in patients receiving standard of care chemotherapy. Durvalumab strips away a defense used by cancer cells, exposing them to attack by killer T cells. Motolimod, meanwhile, activates a protein called Toll-like receptor 8 (TLR8), which is found in a variety of immune cells and serves as an alarm for the frontline forces of the immune response.

The expectation is that Motolimod's activation of TLR8 will create conditions within tumors that are optimal to enhancing the effects of durvalumab. Further, given with chemotherapy, Motolimod might "The Clinical Trials Management operation has a gazillion little wheels. It is a giant project."

additionally boost anti-tumor responses by helping the immune system better "see" the molecular signs of cancer. Together, it is hoped, the therapies might decimate the most resistant of ovarian tumors.

Old's endlessly characterized NY-ESO-1 has not been forgotten either. Ludwig and CRI have tested their cancer vaccines in combination with a checkpoint blockade and are preparing to expand this combination to a pair of checkpoint blockade therapies. That's in addition to efforts by many researchers and institutions to devise their own NY-ESO-1-related therapies based on the work done by Ludwig and CRI researchers.

In any case, there's no shortage of immunotherapeutic pathways for Ludwig and CRI to explore and exploit. "The question in the field now is how to expand that proof of concept we've obtained for immunotherapy in such cancers as melanoma and kidney and lung cancer to a broader variety of cancer types and patients," says Kolom. "To get to where we want to be for the next generation, where we have the right drug picked for the right patient, we have to have a much more sophisticated understanding of what to look for in the tumor's interaction with the immune system. Data from the Ludwig-CRI trials will provide the road map for that endeavor." 占

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