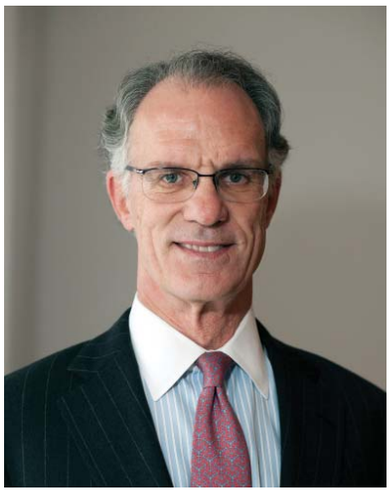
NEW PATHS OF DISCOVERY

2012 RESEARCH HIGHLIGHTS

LUDWIG CANCER RESEARCH

FROM THE PRESIDENT AND CEO



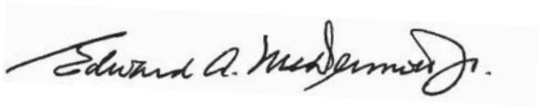
Ludwig scientists work relentlessly to accelerate scientific breakthroughs and save lives. Every day they're engaged in life-changing science — probing ways to prevent and detect the disease in its earliest stages when the warning signs are often subtle and can be missed, combining cancer immunotherapy drugs in patients with advanced melanoma to attack tumors more effectively or developing drugs to take aim at new targets in brain cancer. As the stories in this year's annual report reveal, their groundbreaking research is making real progress, expanding our knowledge and bringing us closer to better diagnostic tools and treatments for the disease.

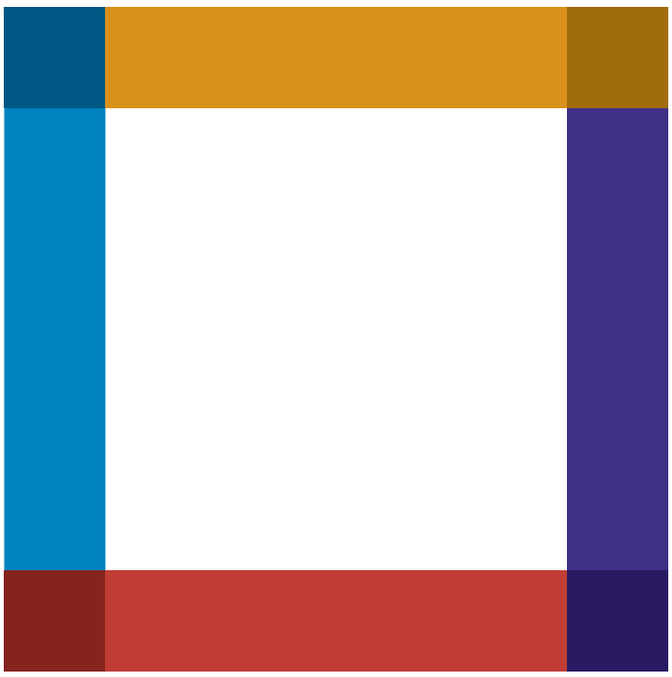
Mr. Ludwig would be proud of the organizations that he bequeathed his fortune to and that bear his name. The Ludwig Institute and the six Ludwig Centers, collectively Ludwig Cancer Research, represent one of the most potent forces today in finding smarter ways to defeat cancer. His extraordinary generosity has brought together some of the best and innovative thinking in cancer research. The wealth of experience and diverse perspectives of Ludwig scientists ensure that we continue to forge new ground in cancer.

Mr. Ludwig was a tough taskmaster. He was demanding, impatient and expected perfection. He was relentless in pursuit of his objectives and intuitively understood that having an impact requires taking risks. Much like our scientists, he appreciated that conquering a disease as complex as cancer would be incredibly difficult.

Many challenges lie ahead, but our scientists are eager to meet them. They are poised to continue to answer critical scientific and clinical questions and make game-changing contributions to the development of cancer treatments. The innovative work this past year has unlocked new paths of discovery and pushed back uncharted frontiers.

Edward A. McDermott Jr.
President and CEO
Ludwig Institute for Cancer Research





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PREVENTION

Most cancer research focuses on curing advanced cases, but the biggest gains against the disease may be in learning how to prevent it. Key to this goal is developing new ways to detect cancer at its earliest stages, an approach Ludwig researchers have embraced.

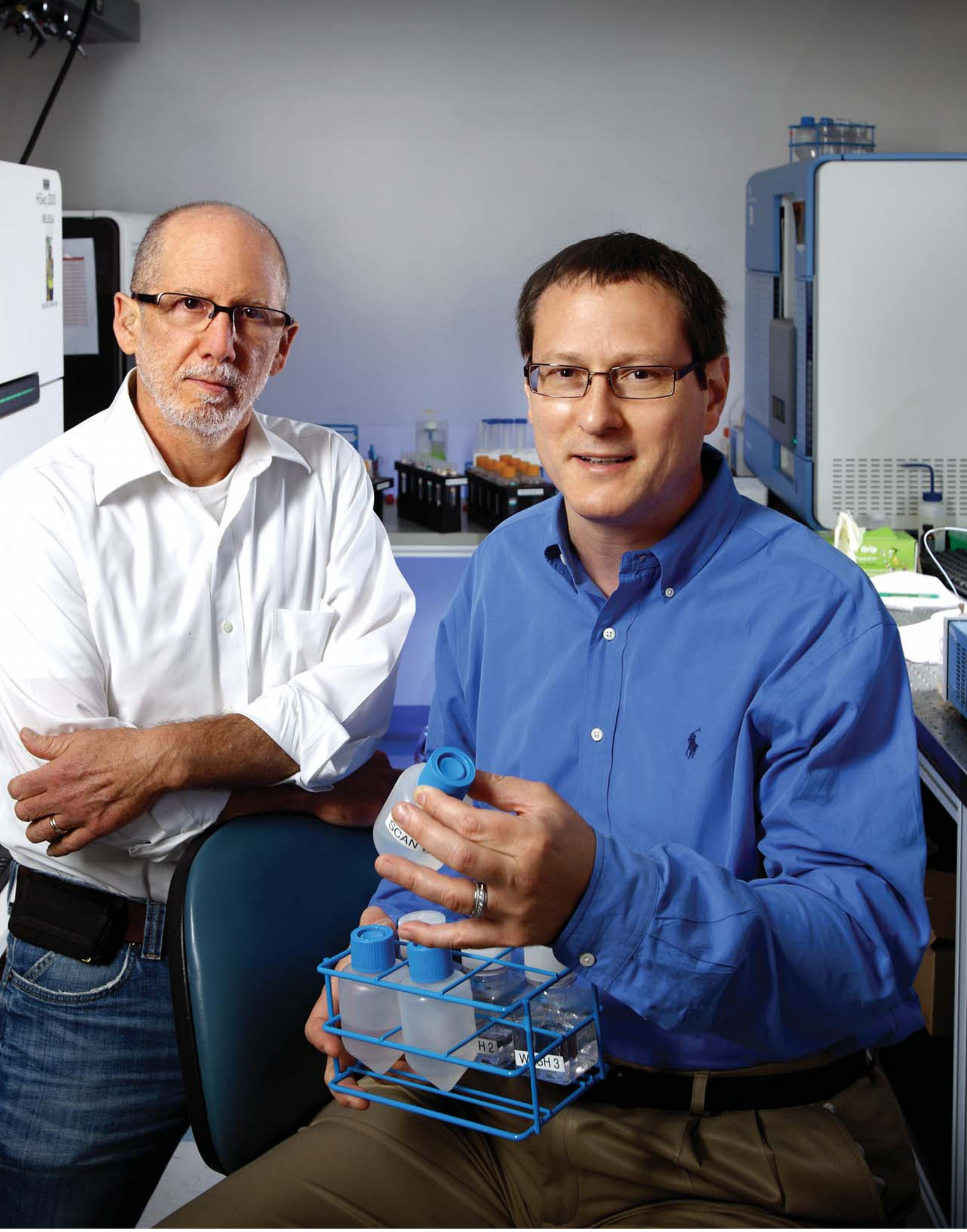
TO CATCH A KILLER

Georgios Papanicolaou invented one of the first tools to detect cancer at its earliest stages, the Pap smear. When he made his discovery in 1928, Papanicolaou knew that early detection could save the lives of women with cervical cancer.

Now, many decades later, the Pap smear is used routinely to test millions of women each year. But Papanicolaou also saw even further into the future. He speculated that the procedure could be used to detect cancers of the uterus and ovary because cells would slough off of their tissues into the Pap smear.

Last year Bert Vogelstein, Ken Kinzler and their colleagues at the Ludwig Center at Johns Hopkins put Papanicolaou's idea to the test. The result is the PapGene test, a genetic approach to detecting ovarian and endometrial cancers using material collected during a routine Pap smear. If the test is successful in large studies, it could put a dent in the more than 200,000 deaths caused annually worldwide by these two cancers. The team's findings also showcase Ludwig's commitment to supporting research focused on early cancer detection and prevention — a major effort funded in part by the Conrad N. Hilton Foundation.

"Many of the gains in cancer research in the next decades will come from early detection and prevention, rather than from curing advanced cases," says Vogelstein.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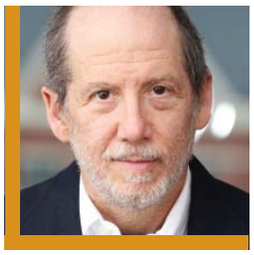

Bert Vogelstein

Ken Kinzler

"Many of the gains in cancer research in the next decades will come from early detection and prevention rather than from curing advanced cases."

BERT VOGELSTEIN

The PapGene test emerged from a deep understanding of the mutations that drive cancer, which has long been a research focus at Ludwig Johns Hopkins. In their study, the researchers assessed Pap smears to detect DNA mutations associated with uterine or ovarian cancer. They successfully identified cancer in all the women in the study with endometrial cancers, and in 41 percent of those with ovarian cancer. From these initial experiments, they developed the PapGene test, a sequence-based assay that detects mutations in 12 genes frequently altered in these cancer types.

The researchers are now assessing how well the PapGene test works in a larger sample of patients. And they are investigating ways to better detect ovarian cancer, such as tweaking the device used for the Pap smear to collect more ovarian cancer cells.

Kinzler says researchers throughout the world have invited him to collaborate. "People are really excited about this," he says. "This is something that hopefully can be implemented in the clinic in the foreseeable future."

> "We named our test PapGene in honor of Papanicolaou. He pioneered the idea that noninvasive tests for cancer can save lives if widely implemented." **KEN KINZLER**

In a second ongoing project, Vogelstein, Kinzler and their colleagues are investigating ways to detect cancer by analyzing DNA in the bloodstream. Dying tumor cells release a small amount of DNA into the blood, and this DNA can be detected using exquisitely sensitive technologies developed by the group. Most recently, they designed ways to comprehensively analyze tumor DNA for changes in chromosomal copy number and DNA rearrangements, alterations that occur in most tumors. They used this approach to detect cancers by examining blood from ten late-stage colorectal and breast cancer patients. The method could enable the noninvasive detection of nearly all cancer types.

DNA sequencing technology is still too expensive for everyday use. But costs are decreasing rapidly each year. Vogelstein is confident that at least one of the blood tests their group has developed will ultimately be inexpensive enough for routine clinical use. He and Kinzler have helped found the biotechnology company Inostics to carry this approach forward.

Vogelstein and Kinzler hope their research will one day have as much of an impact on cancer as Papanicolaou's work. "We named our test PapGene in honor of Papanicolaou," says Kinzler. "He pioneered the idea that noninvasive tests for cancer can save lives if widely implemented."

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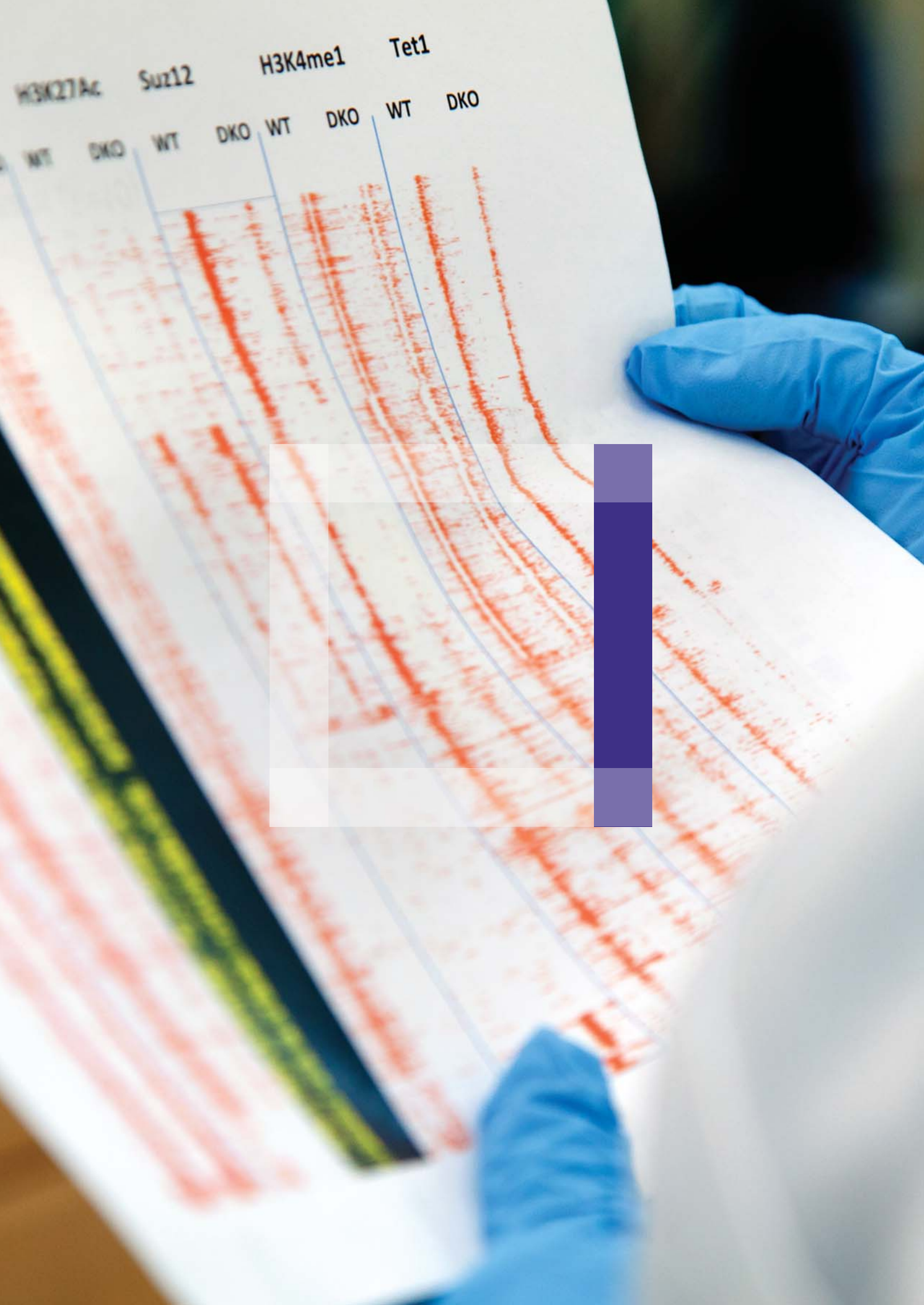
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TECHNOLOGY

New scientific technologies are the wellspring for major research discoveries, and they often lead to new diagnostic and therapeutic tools. Such connections are coming alive in the laboratories of innovative Ludwig researchers.

DECODING THE HUMAN GENOME

When Bing Ren interviewed for a position at Ludwig San Diego in 2001, his scientific discipline was just emerging. Researchers were beginning to explore how chemical modifications to DNA, or the proteins that bind to it, could change how genes are regulated.

They were looking at these modifications throughout the genome. This area of study, called epigenomics, was about to take off, “Biology was set for a major conceptual shift,” recalls Web Cavenee, Ludwig San Diego director.

At the time, much research in cancer biology focused on how a single gene or molecular process affects a tumor cell. But Ren was thinking bigger. He was examining the genome of a cell as a unit, and assessing how entire sets of genes are turned on or off. He was particularly good at this approach, having developed a key technology called ChIP-on-chip. This technique identifies regions of DNA bound by proteins that help determine whether a gene is active or silent. By illuminating where on DNA such epigenetic changes occur, Ren’s approach could help researchers understand how genes are regulated, cells proliferate and disease progresses.

“His work struck me as intellectually daring and was coupled with flawless experimental controls,” recalls Cavenee. But there was a flip side. “I knew that work like this, taking him beyond the edge of knowledge, was also likely to have a hard time being funded.” Cavenee decided to take a leap and hire Ren. Neither researcher has been disappointed.

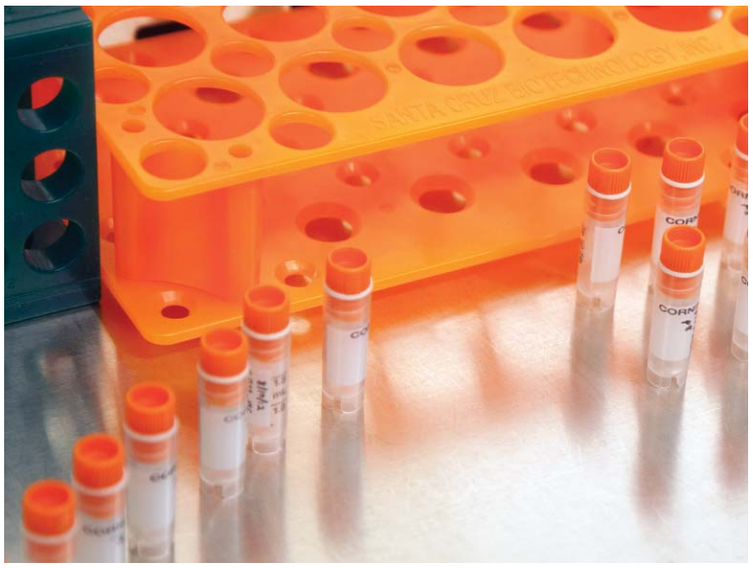
“Joining Ludwig was the best decision I have ever made in my career,” says Ren. He has since become a leader in epigenomics. In 2012 his laboratory produced four major studies revealing how cells manage the activity of their genes, for instance turning them on or off depending on cell type or signals from the environment. In the long term, the research could lead to new technologies to diagnose and assess tumor types. Ludwig’s initial investment

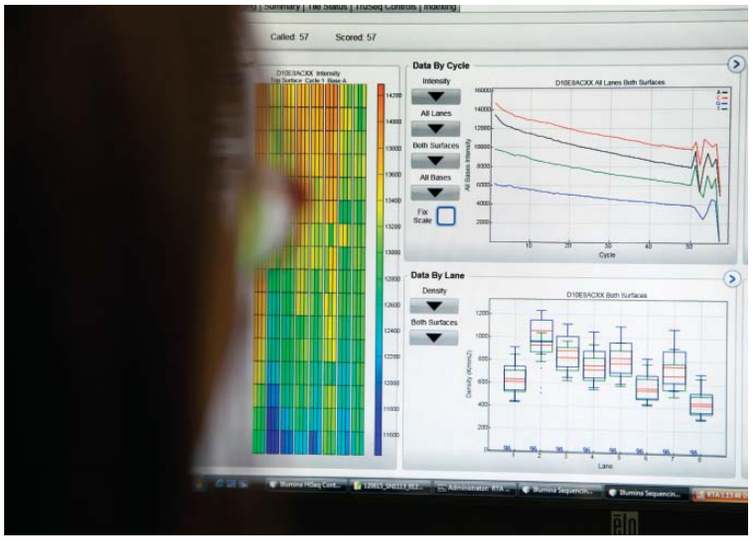
Bing Ren

has snowballed: Ren's lab is now a center for two large international research initiatives, patterned after the human genome project, to assess gene regulation in normal and diseased cells.

Ren's laboratory is one of seven centers for the ENCyclopedia of DNA Elements (ENCODE) Project, which is funded partly by the US National Institutes of Health. The project aims to catalog DNA sequences that regulate whether genes are expressed. And another major initiative, the NIH Roadmap Epigenomics Project, has tapped Ren's laboratory to run one of its four centers. His focus is analyzing the epigenomes of embryonic stem cells to map key modifications to DNA and histones, proteins that bind to DNA.

Ren traces his bounty of recent data to a moment in 2007 when he realized that his lab might be falling behind. He had just learned of new DNA sequencing technology that could fast-track ChIP-on-chip, enabling the technique to examine DNA faster and more comprehensively. The "next generation" DNA sequencing machines had the potential to accelerate Ren's research dramatically, but they cost a hefty $750,000 each.





"Joining Ludwig was the best decision I have ever made in my career." **BING REN**

Such a big purchase would normally require its own grant, which could take a year to work its way through most institutions.

But Ludwig was able to corral the resources. Within a week, the funding was secured, and Ren had his machine. "These four studies are a direct result of our early access to next-generation sequencing technologies," says Ren.

Each of the studies focuses on a different aspect of gene regulation. But they all stem from the same fundamental idea—that cancer and other human diseases can arise not only from mutated genes, but also from defects in how those genes are turned on and off.

Two of the studies emerged as part of the ENCODE Project. In one of last year's international scientific triumphs, the project coordinated the release of almost 100 studies. Ren and his colleagues contributed a study showing how DNA is organized into domains that tend to fold together, promoting interactions between genes and their regulatory sequences. In a second study, they identified the location of nearly 300,000 DNA regulatory sequences, covering about 11 percent of the bases of the mouse genome. A third study, which was part of the NIH Epigenome Project, deployed a new technique to identify the locations of a key type of DNA modification, 5-hydroxymethylcytosine, which abounds in human and mouse brains and in embryonic stem cells.

Ren is already applying his technique to understand the epigenomics of cancer. In a fourth study in 2012, he teamed up with Ludwig researchers in Baltimore, Lausanne, New York and São Paulo. The researchers examined the cancer epigenome, the sum of modifications to DNA or histones that may affect the expression of cancer-related genes. They released a study of breast cancer showing how low levels of a DNA modification called methylation lead to DNA silencing.

“How does a normal cell become metastatic by acquiring the ability to grow indefinitely, or evade the immune system, or become impervious to dying?” asks Cavenee, who is now teaming up with Ren to study the epigenome of glioblastoma, a deadly brain cancer.

Ren’s work on fundamental aspects of gene regulation in normal and cancerous cells is already beginning the journey to the clinic. His research also may one day complement the work of other Ludwig researchers, such as Bert Vogelstein, who design tools to detect and evaluate tumors at their earliest stages, when cancer is easiest to cure. Ren’s research could eventually lead to new, affordable diagnostic tools for tumor assessment.

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SEQUENCING AND THE SINGLE CELL

Across the globe from San Diego, where Bing Ren and his colleagues hone techniques to assess gene activity, a Ludwig laboratory in Stockholm is perfecting another technology to bolster cancer research.

Rickard Sandberg

Ludwig scientist Rickard Sandberg leads a team that is developing Smart-Seq, a method to assess genes at the level of a single cell. The method, which Sandberg published last year, could address many difficult questions in tumor biology, such as the fate of individual cells that break off a tumor and enter the circulation, and how cells within a tumor differ from each other. “This technique provides a sensitive and detailed investigation of a single cell,” says Sandberg.

Molecular biologists generally combine thousands or millions of cells in a test tube to obtain enough cellular material to perform experiments. But tumors do not consist of uniform cells. On the contrary, cellular diversity is thought to contribute to cancer development. For instance, cancer cells with different types of mutations or different sets of active genes can cooperate with each other or with supporting cells to foster tumor growth. Moreover, sometimes a rare cell within a tumor withstands cancer drugs and seeds the regrowth of a tumor after therapy. But such important processes are hard to study because it is difficult to assess the molecular state of individual cells.

To overcome this barrier, researchers have for years been attempting to shrink their techniques, tweaking molecular biology protocols and designing specialized equipment to handle single cells. Last year, Sandberg and his colleagues, including researchers at the genomics company Illumina, hit on a winning combination in Smart-Seq. The technique can scan a single cell for DNA mutations, including mutations that might contribute to cancer, and determine which genes are turned on or off in a cell—all more effectively than previous methods. The team’s findings were published in *Nature Biotechnology*.

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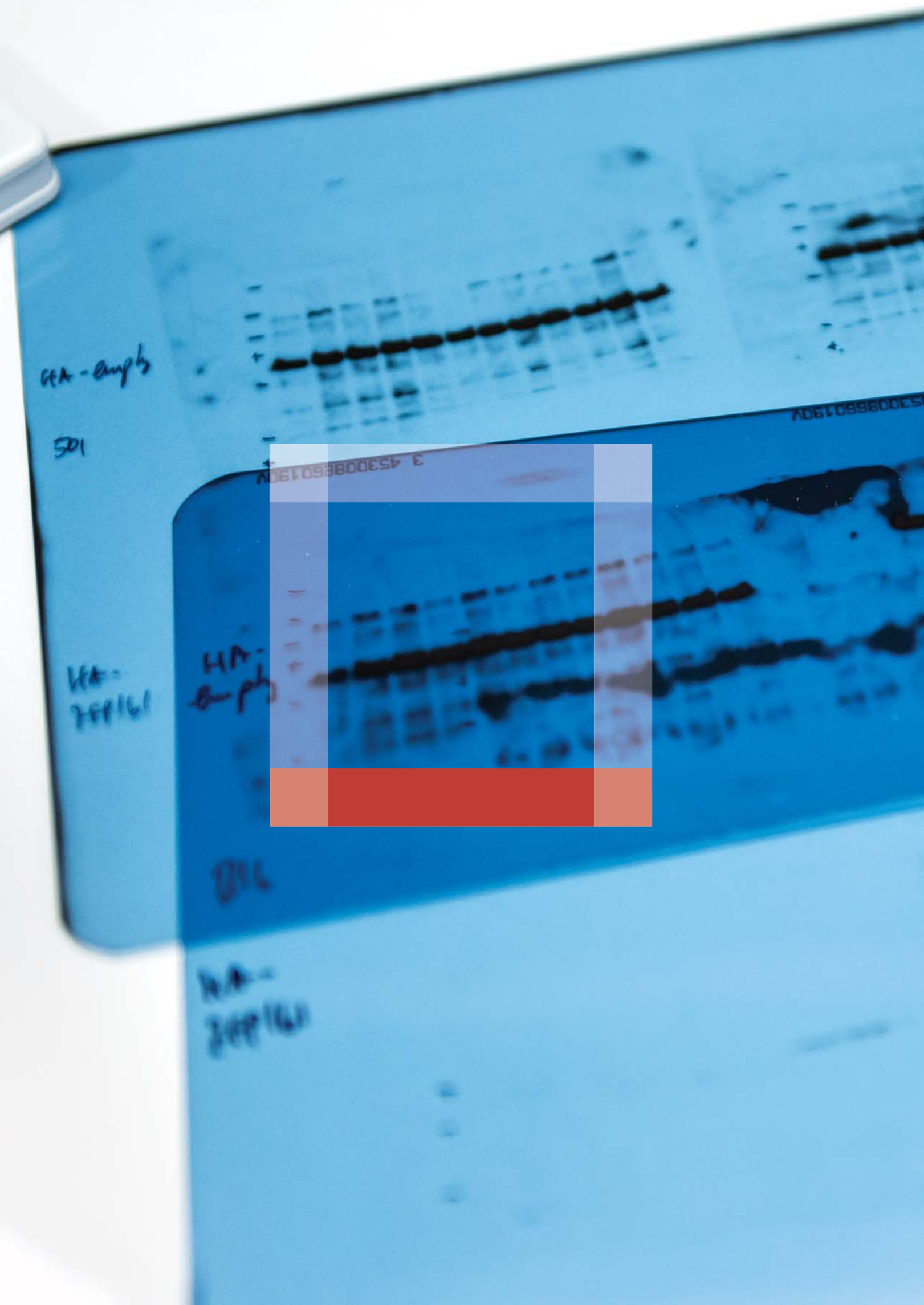
> "This is a pilot study but it provides a very concrete example of how the technique could be important for clinically relevant cells." **RICKARD SANDBERG**

In the study, the researchers examined single melanoma cells plucked from the bloodstream of a melanoma patient. They identified genetic mutations associated with cancer in these rare cells and assessed genes that are aberrantly regulated.

"This is a pilot study," says Sandberg, "but it provides a very concrete example of how the technique could be important for clinically relevant cells." In the future he hopes to apply the technique to questions such as how cancer cells change as they break off from a primary tumor and enter the circulation, what kinds of molecular changes enable them to survive, and how some of them lodge at distant sites to seed a new tumor.

Sandberg is still refining the technique to make it faster, less expensive and ready for routine use. Ludwig supports Sandberg with the same long-term view it has taken on Bing Ren's research, and Sandberg is confident he will soon succeed.



INTERDISCIPLINARY RESEARCH

Ludwig Cancer Research has a long tradition of nurturing investigators who see connections among different fields. The work of Ludwig scientist Don Cleveland shows how research in neuroscience is leading to new approaches to treat brain cancer.

TECHNIQUE TO VANQUISH DISEASE IN NERVOUS SYSTEM APPLIED TO CANCER

Richard Smith, a neurologist who has a roster of patients with neurodegenerative disease, would not leave Ludwig scientist Don Cleveland alone. Cleveland's laboratory at Ludwig San Diego had the means to test an idea to help people with amyotrophic lateral sclerosis (ALS, also called Lou Gehrig's disease), whom Smith saw regularly at his San Diego clinic. "He was relentless in badgering me," recalls Cleveland of their interactions in 2005.

Don Cleveland

The idea was to silence a gene that is mutated in a proportion of inherited ALS cases, caused by mutation in SOD-1 (superoxide dismutase 1), in the hope that quelling the bad gene could also quell disease. To silence the gene, Smith suggested using an antisense oligonucleotide, a small piece of single-stranded DNA. It was designed to destroy the gene's mRNA, a molecular intermediary between the gene and the protein it encodes. Smith proposed testing the oligonucleotide in animals with a pump that would infuse the DNA drug directly into the cerebrospinal fluid, essentially bathing the brain and spinal cord in it.

To work, the drug needed to be taken up by the motor neurons. Cleveland didn't think this would happen. But Smith, who later spent two years in Cleveland's lab working on the project, argued that they had nothing to lose. "He was so tired of having nothing to offer to his patients," says Cleveland. People with ALS suffer from increasing muscle weakness followed by paralysis, and they often die within a year or two of diagnosis.

“Ludwig allows its investigators to go in directions that they think can make a positive contribution.”

DON CLEVELAND

To Cleveland’s surprise, the procedure was successful, slowing progression of ALS-like paralysis in a mouse harboring the mutant gene. When the researchers examined the nervous system of treated mice and monkeys, they observed that the oligonucleotide had found its way into all the crevices and corners of the brain and spinal cord, knocking out the mutant gene. Cleveland knew then that they had something big on their hands. “A light bulb went off,” he says. “I realized this might be more broadly useful for a range of human diseases.”

Since those first experiments, Cleveland’s lab, together with industry partner Isis Pharmaceuticals, has branched out. He and his team have applied their technique to a variety of conditions. Last year they completed animal studies on the neurodegenerative condition Huntington’s disease. And they are currently testing their technique in mouse models of glioblastoma, the most common and aggressive form of brain cancer. The researchers already have laid groundwork for moving into testing of cancer patients should the animal experiments be successful. Their technique was shown to be safe in phase 1 clinical trials for ALS, and is now being tested in people with three other conditions, including the genetic disorder spinal muscular atrophy.

The group's study on Huntington's disease, which was published in *Neuron*, shows how powerful the technique can be. The researchers infused an oligonucleotide into mice and monkeys to silence huntingtin, the gene mutated in Huntington's disease. In a mouse model, symptoms of the disease continued to improve for months after a single two-week infusion, long after the drug had begun to disappear from the nervous system. And in primates, a 21-day infusion reduced huntingtin mRNA in most brain and spinal cord regions. The effect was sustained for at least four weeks after treatment. This strong, long-term effect bodes well for the technique's potential to treat other conditions.

To test whether the approach will work for glioblastoma, Cleveland is developing oligonucleotides against several genes, including *CenpE*, which is involved in separating chromosomes during cell division. Cleveland's lab discovered *CenpE* more than 20 years ago while studying how chromosomes are parceled out during cell division.

Interfering with *CenpE* can stop glioblastoma cells from dividing without destroying normal neurons, a drawback of many conventional chemotherapy drugs. The group's pump system should also help overcome another obstacle to cancer therapy—getting drugs into the brain, which is shielded by the blood-brain barrier. Meanwhile, Cleveland and his colleagues are developing potentially even more effective nucleotide therapies based on single-stranded RNA, an approach they published last year in *Cell*.

Web Cavenee, director of Ludwig San Diego, says that Cleveland's overlapping interests in chromosomes, cancer and neurodegeneration are a boon to Ludwig. "His studies provide an entry into cancer that would not have been taken by a more conventional cancer-centric approach."

Cleveland credits Ludwig with recognizing that his research on neurological diseases could generate a breakthrough for cancer.

"Ludwig allows its investigators to go in directions that they think can make a positive contribution," says Cleveland. "The idea that everything needs to be highly directed to solve major questions in human disease is just not true and the leadership of Ludwig understands that."

"I realized this might be more broadly useful for a range of human diseases."

DON CLEVELAND

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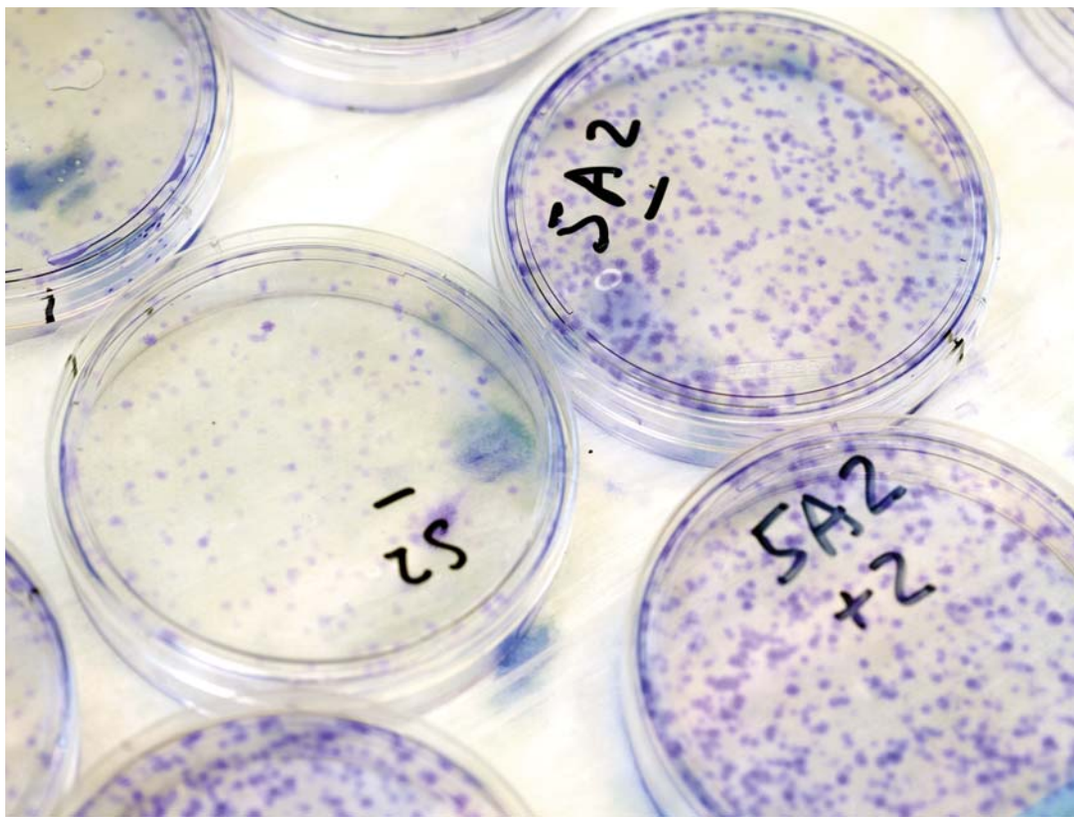


COMBINATION

Ludwig Cancer Research has long fostered research in several core areas. These include the study of melanoma, brain tumors and, more broadly, immunotherapy—research on how to modulate the immune system to attack tumors. Ludwig's emphasis on these core areas has led to a multipronged view of tumors, fostering the development of therapies that can be used in combination. This approach may counteract cancer more effectively than any single treatment used in isolation.

TAPPING INTO NEW TARGETS FOR BRAIN CANCER

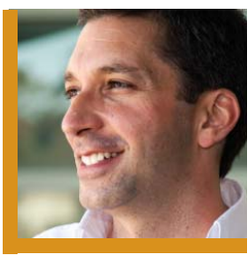
If any tumor type is a candidate for combination therapy, it is glioblastoma multiforme. This aggressive brain cancer defies most treatment strategies. Chemotherapy barely touches it, and drugs that target cancer-causing cellular molecules are also remarkably ineffective in treating it. Median survival is 15 months after diagnosis.

Ludwig has long supported research programs to tackle this disease. Recently, physician-scientist Paul Mischel, a former faculty member at the University of California, Los Angeles, was recruited to the Ludwig San Diego team. He has helped design and lead molecular analysis in five clinical trials of therapies targeting cancer-related cellular molecules. Mischel's expertise complements a team with strengths in basic research. It includes geneticist Frank Furnari and Ludwig San Diego director Web Cavenee, who study the diverse cellular mechanisms that drive cancer.

Mischel's move to Ludwig last fall was a natural evolution of an ongoing long-distance interaction with Furnari and Cavenee. "We have shared interests and complementary approaches," says Mischel. "The synergies among us were so great that it made sense to work closer together."

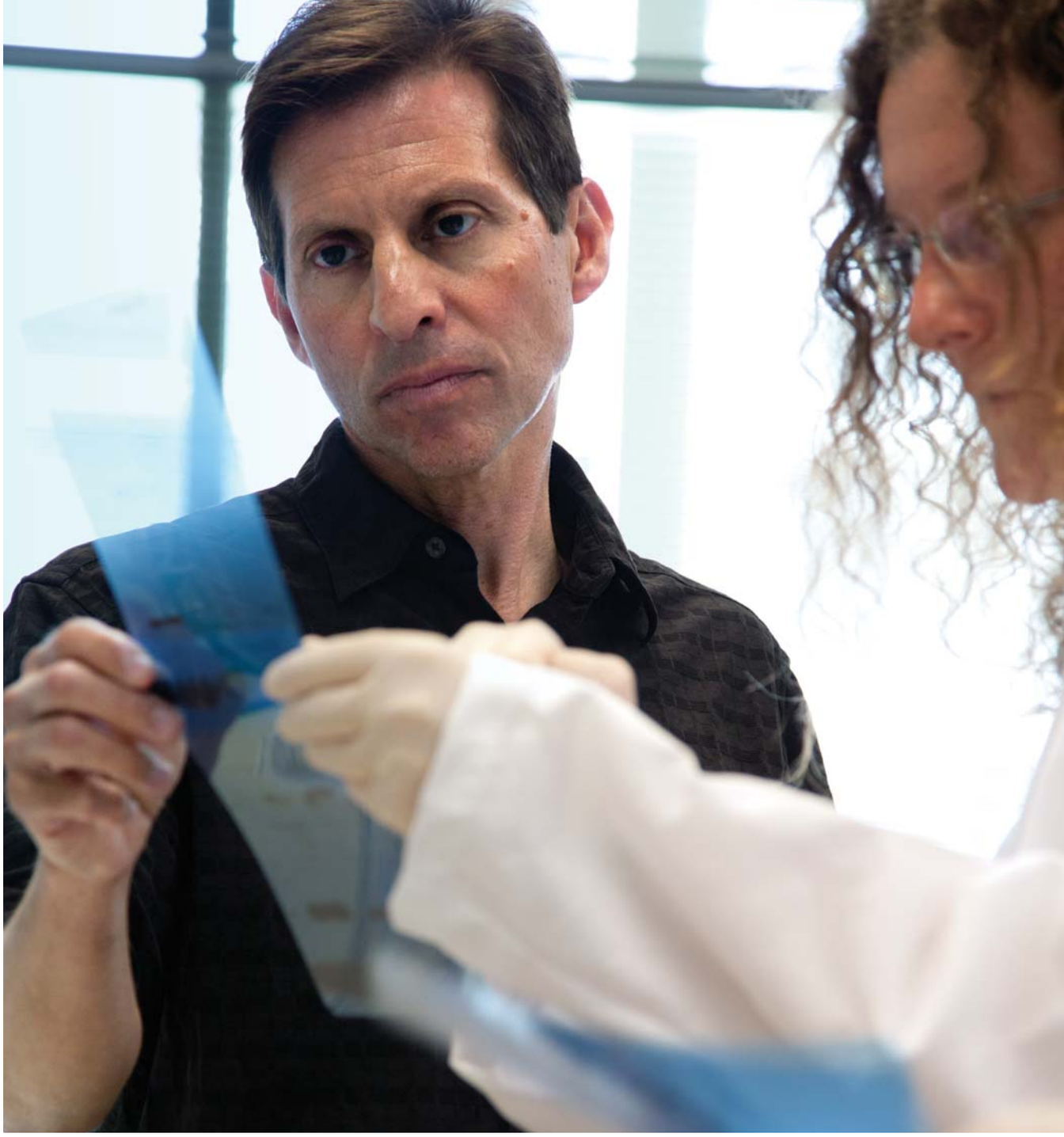
One of the trio's most recent projects delved into why two drugs designed to inhibit a cellular molecule called epidermal growth factor receptor (EGFR) work poorly in patients with glioblastoma. EGFR inhibitors such as these two, erlotinib and gefitinib, are effective in many patients with breast and other cancers. But less than 10 percent of glioblastoma patients respond to the drugs, and when they do, the response is usually short-lived. In figuring out why this happens, the researchers hope to find new ways to combine EGFR inhibitors with other agents to treat glioblastoma and other cancers.

Paul Mischel



Frank Furnari

Web Cavenee



The new study built on previous research pioneered by Mischel in partnership with Cavenee and Furnari. The three have shown that many people with the tumor have defects in a molecule called PTEN, enabling tumor cells to circumvent the drugs. In some unresponsive patients the gene encoding PTEN is mutated. But other unresponsive patients have normal PTEN, suggesting that the molecule can be deactivated in another way.

Last year, the team discovered a new mechanism of PTEN deactivation. They found that the molecule can be inactivated by a molecular modification — the addition of a phosphate molecule. Testing clinical samples obtained by Mischel and Ludwig collaborators in São Paolo, Brazil, the researchers found that PTEN phosphorylation is associated with resistance to EGFR inhibitors and shortens overall patient survival. Experiments on cancer cells in a test tube similarly showed that PTEN phosphorylation leads to resistance to EGFR inhibitors.

The team also identified some of the cellular regulators that add a phosphate group to PTEN. They are now identifying regulators that remove the phosphate group. The researchers ultimately aim to test whether interfering with one of these regulators, for instance targeting them with a small drug-like agent, could reactivate PTEN in tumors. Such an agent could form the basis of a drug to be used in combination with an EGFR inhibitor. The findings were published in *Proceedings of the National Academy of Sciences*.

"We need to fully understand how this mechanism works, and we're hoping these new findings will move us into clinical trials," says Cavenee. This is something Mischel, with his extensive experience in the clinic, is well prepared to do in collaboration with his new San Diego colleagues. Says Cavenee, "We are constantly walking into each other's offices and bouncing ideas off of one another. I should have hired Paul years ago—but better late than never!"

"We need to fully understand how this mechanism works, and we're hoping these new findings will move us into clinical trials." **WEB CAVENEE**

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"We are constantly walking into each other's offices and bouncing ideas off of one another. I should have hired Paul years ago—but better late than never!" WEB CAVENEE

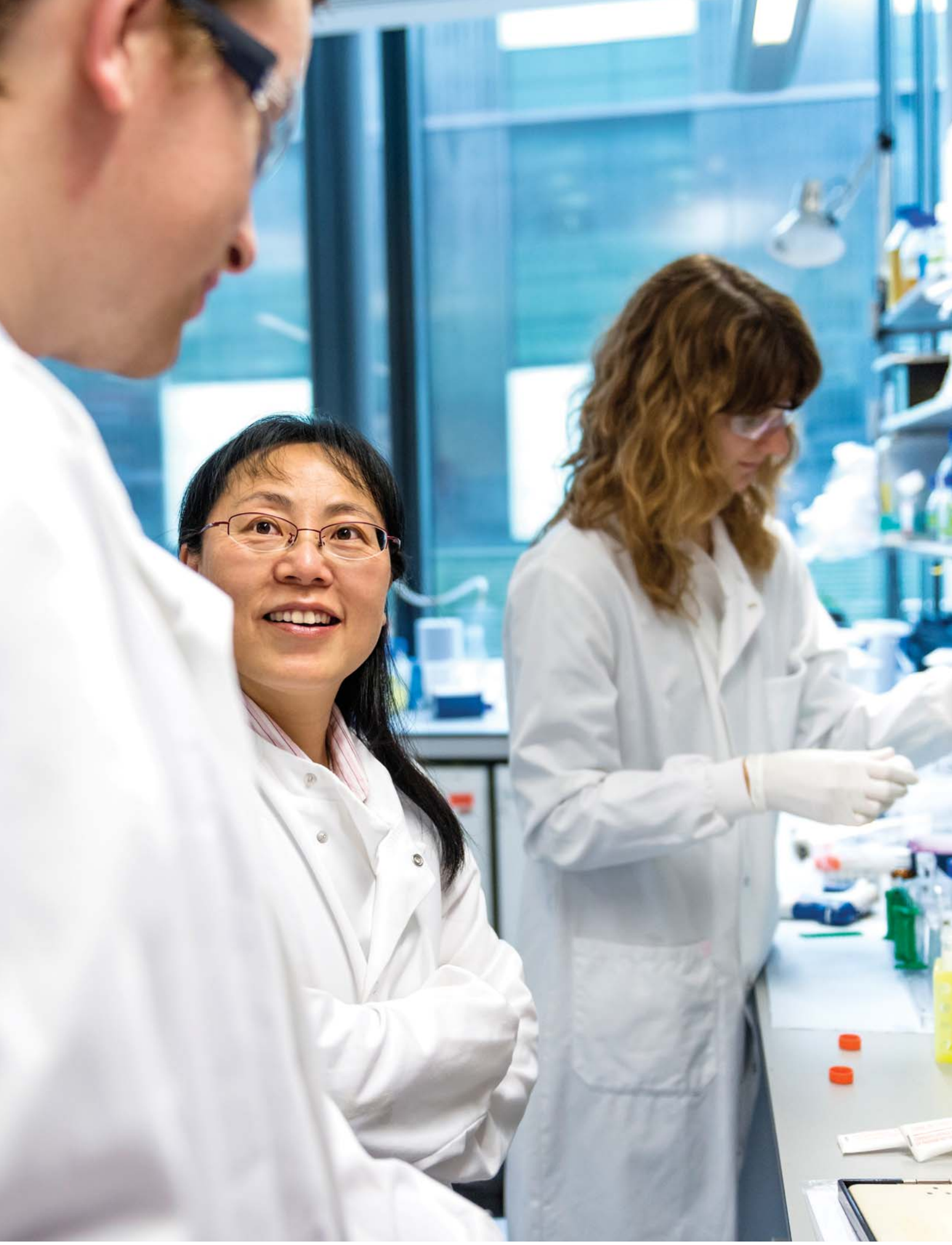
THREE DRUGS ARE BETTER THAN ONE

Cancer treatment is rapidly undergoing a shift toward “targeted” drugs, which are designed to act on specific molecules that drive cancer or modulate its growth and metastasis. But these drugs have not all lived up to their original promise; they often only temporarily fend off disease.

One such drug is vemurafenib. It targets a molecule found in more than half of melanoma cancers, a mutant form of the protein B-RAF, which fuels cancer by instructing cells to divide. Vemurafenib inhibits mutant B-RAF, and it can have dramatic effects in melanoma patients, shrinking tumors throughout the body. But the effects of vemurafenib are transient. Tumors invariably roar back, often within several months. Xin Lu, director of Ludwig Oxford, is working on a solution to this issue: combination therapy.

“If we could target a parallel pathway we could potentially create a better therapy,” says Lu. She is conducting experiments combining vemurafenib with drugs targeted to a parallel pathway involving p53, a molecule that puts the brakes on tumors. p53 is nonfunctional in many tumors, including about 90 percent of melanomas. Lu’s approach is to “wake up” p53 with experimental drugs, enabling it to arrest cancer.

Although experimental drugs to reactivate p53 have been developed previously, they are not as effective as researchers had hoped. Lu went back to the laboratory bench to identify molecular regulators of p53 and find new ways to target this molecule. After years of research, she landed on a protein



> “If we could target a parallel pathway we could potentially create a better therapy.” **XIN LU**

called iASPP that binds to p53 and modulates its actions. She and her colleagues then developed a drug-like agent that targets iASPP and wakes up p53. The new agent comes with an added bonus: it synergizes with a previously developed drug that targets p53. Together, the two agents activate p53 more powerfully than either one alone.

The researchers tested these two p53-activating agents in combination with vemurafinib. They observed that the triple combination had a strong effect, killing melanoma cells in culture and resulting in sustained tumor shrinkage in mice. The findings, published in *Cancer Cell*, have implications beyond melanoma. The two p53-activating agents, for instance, could be used in combination with other targeted drugs.

For this project, Lu worked together with her Oxford colleagues, as well as Ludwig melanoma researchers in Melbourne and Baltimore. Lu also credits Ludwig with providing the stable funding needed for the years of basic research that led to this study. “That stability allowed me to tease out the whole difficult molecular pathway involving iASPP,” she says.

Meanwhile, Lu continues to untangle the complicated molecular networks that propel tumors. In 2012 she published a study on the mechanics of another protein that binds p53, a molecular relative of iASPP that helps drive tumors into a dormant state. With time, this work could also lead to new approaches to treat cancer.

BACK TO THE BASICS

Ludwig's long-term commitment to basic research propels the science necessary to replenish the drug pipeline at its earliest stages. Its strong support of the work of Xin Lu in Oxford is mirrored in Uppsala, Sweden, in the laboratory of Ingvar Ferby, who was recently recruited to Ludwig. Ferby is dissecting the molecular pathways that impinge on epidermal growth factor receptors (EGFRs). EGFRs are a target of cancer drugs such as gefitinib, which shuts down the receptor. The effectiveness of such drugs could be bolstered by the development of combination therapy.

Last year Ferby identified a molecular pathway that prompts cell death—a blow to cancer—when EGFR is shut down. The pathway involves a protein, Mig-6, that binds to the receptor. When the receptor is inactive, Mig-6 initiates cell suicide. The findings could lead to new targeted anticancer agents to tweak cellular processes such as cell death. Some of these agents might be useful in combination with drugs such as gefitinib.

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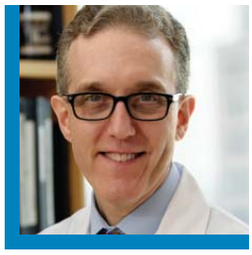
QUELLING MELANOMA WITH IMMUNOTHERAPY

When Ludwig scientist Jedd Wolchok looked at the radiographs of his patient, a 42-year-old woman with advanced, recurrent metastatic melanoma, he was stunned. Where there should have been tumors, there were none.



Jedd Wolchok

His patient, a single mother of three, had already gone through several rounds of treatment. After her chemotherapy failed, she took the melanoma drug ipilimumab, which powers up the immune response to cancer. While she was on the drug, her tumors continued to grow, albeit more slowly than before, and new ones began to appear in other organs. Then she received palliative radiation to quell the pain from a tumor pressing on nerves exiting her spine. Three months later, her radiographs showed that the treatment had not only shrunk the tumor near the spine, as expected, but also minimized or eliminated many of the other tumors in her body.

"I had never witnessed this phenomenon," recalls Wolchok. But he knew what it was. There were several reports in the scientific literature describing similar but extremely rare events. In what is called the "abscopal effect," radiation directed at one tumor in the body affects tumors far from the site of the treatment. "This was the most important scientific surprise of the year for us," says Wolchok.

Wolchok and his colleagues went back to the patient's blood samples to try and piece together what had happened. Could ipilimumab, a new drug on the market, have had something to do with the patient's response to radiation?

The blood workup revealed the outlines of an explanation. Before she was treated with ipilimumab, the patient had an immune response to a protein dubbed NY-ESO-1. It is a tumor antigen that is almost exclusively associated with tumor cells. Ipilimumab seems to work better in patients, such as this one, with pre-existing immunity to NY-ESO-1. Previous studies had shown

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that the drug increases the immune response to this tumor antigen and other cancer-associated molecules. But in this case the radiation multiplied the effect. Radiation seems to have caused dying melanoma cells to release new target antigens, as well as changes in the tumor microenvironment that fostered tumor destruction. “The immune system’s cancer-fighting response was turned up,” says Wolchok.

Ten months after radiation treatment, the patient was stable and her remaining tumors, if any, were still tiny.

The findings, published in the *New England Journal of Medicine*, have led to several clinical trials to test ipilimumab in combination with various radiation regimens. One of these trials, led by Wolchok and managed by Ludwig clinical trials experts in New York, will soon begin in collaboration with Ludwig researchers at Memorial Sloan-Kettering Cancer Center, the University of Chicago and Stanford. Throughout the trial, subjects’ immune systems will be closely monitored so the researchers can better understand how these two treatments synergize. The data could lead to more effective ways to combine immune agents like ipilimumab with therapies such as radiation or chemotherapy.

Wolchok’s studies highlight a major theme of cancer research — the power of combination treatments. In addition to pairing radiation with an immune-boosting agent, many other combinations are under investigation, such as combining two or more “targeted” drugs directed against specific molecules associated with cancer cells.

“Two of medicine’s most vexing problems, tuberculosis and HIV, were only satisfactorily controlled when combinations were used,” says Wolchok. “In cancer we are at that same point,” he adds, explaining that combination therapies in this clinical trial and others still being investigated in the laboratory could make a big difference in the lives of patients. This theme has been embraced by Wolchok and other scientists at Ludwig and was a particularly fruitful avenue in 2012.

Wolchok’s laboratory, for instance, is involved in clinical trials combining ipilimumab with a vaccine to bolster the immune response to NY-ESO-1. The group hopes that this will increase the effectiveness of ipilimumab. They are also combining ipilimumab with nivolumab, a new immune modulator. Nivolumab neutralizes the programmed death 1 protein, which cancer cells can exploit to escape destruction by the immune system. Promising results from this study were unveiled in June 2013 at the Annual Meeting of the American Society of Clinical Oncology and in the *New England Journal of Medicine.* The findings from the phase 1 trial showed that a regimen of the two antibody therapies led to strong and durable tumor regression in

> "When OX40 is activated on regulatory T cells in the tumor, they get so stimulated that they actually die." **JEDD WOLCHOK**

patients with inoperable, metastatic melanoma. The researchers have also gone back to the laboratory bench to find new ways to combine immunotherapy treatments to destroy tumors.

In one such study, published in the *Journal of Experimental Medicine*, Wolchok and his colleagues combined conventional chemotherapy with two experimental immunotherapies, a drug-like antibody and a cell-based treatment called T cell transfer. The antibody activates OX40, a molecule on the surface of immune cells, including cells that shield tumors from destruction called regulatory T cells. "When OX40 is activated on regulatory T cells in the tumor, they get so stimulated that they actually die," explains Wolchok. The researchers then explored whether adding T cell transfer to the mix would improve the outcome. This cell-based treatment involves removing T cells from the bloodstream and engineering them to target a molecule on tumor cells, flagging the tumor cells for destruction. The tumor-fighting T cells are then infused back into the bloodstream. The researchers tested this approach in a mouse model of melanoma, and observed a swift response. The treatment eradicated the tumors, even when it was administered several weeks after the tumors began to grow.

Ludwig plans to take this research forward to test a similar approach in people. They are aided in this effort by the Cancer Research Institute, a nonprofit organization devoted to research and development of immune-based cancer therapies, and a long-standing Ludwig partner. Ludwig and the Cancer Research Institute are collaborating with the biotechnology company MedImmune to obtain an OX40 antibody and other agents to use in human trials.

Meanwhile, immunologists at Ludwig are seeking out new ways to turn the immune system against tumors, and other biologists are developing ways to put a wrench in the cellular machinery that drives the proliferation of cancer. These avenues of research could lead to new therapies that may pack a punch in combination with other treatments.

The clinical and laboratory research of Wolchok and other Ludwig researchers is buoyed by Ludwig's rich, decades long tradition of support for basic research and unswerving commitment to the field of immunotherapy. For example, the NY-ESO-1 molecule, which is critical to the immune analysis of the powerful anticancer response in Wolchok's 42-year-old patient, was first described by Ludwig investigators more than a decade ago.

Says Wolchok, "Because of the high quality of science that has been done at Ludwig for many years, we are in a position to do the kind of work we're doing now."

NEW APPROACHES EMERGE FROM WITHIN A TUMOR

New ideas for immunotherapy emerge from Ludwig research labs throughout the world. The development of these ideas is bolstered by infrastructure Ludwig has fostered for decades, such as a Brussels-based tissue bank containing valuable material obtained with consent from patients with melanoma.

Last year, Ludwig Brussels researcher Nicolas van Baren tapped into this infrastructure to uncover a curious facet of melanoma immunobiology. He and his colleagues found that immune structures could develop in metastatic tumors in people with melanoma. These structures, akin to those that develop in lymph nodes, may be a source of immune cells that keep the tumor in check, preventing it from growing or metastasizing further.

Van Baren hopes to learn how to manipulate these structures to prompt their growth or strengthen their immune activity against a tumor. His research could lead to a new way to bump up immune activity against a tumor and add to the pipeline of treatments entering preclinical and clinical testing.

Van Baren's study, which was published in *Cancer Research*, probes the intersection of two long-standing spheres of Ludwig research, melanoma and immunotherapy. Van Baren collaborates with researchers in Oxford, Lausanne and Melbourne as part of the melanoma initiative to study many shared melanoma cell lines and tumors. Says van Baren, "At Ludwig there are many people working on similar topics. This leads to serendipitous interactions that in turn elevate the level of science."

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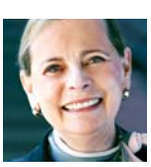

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