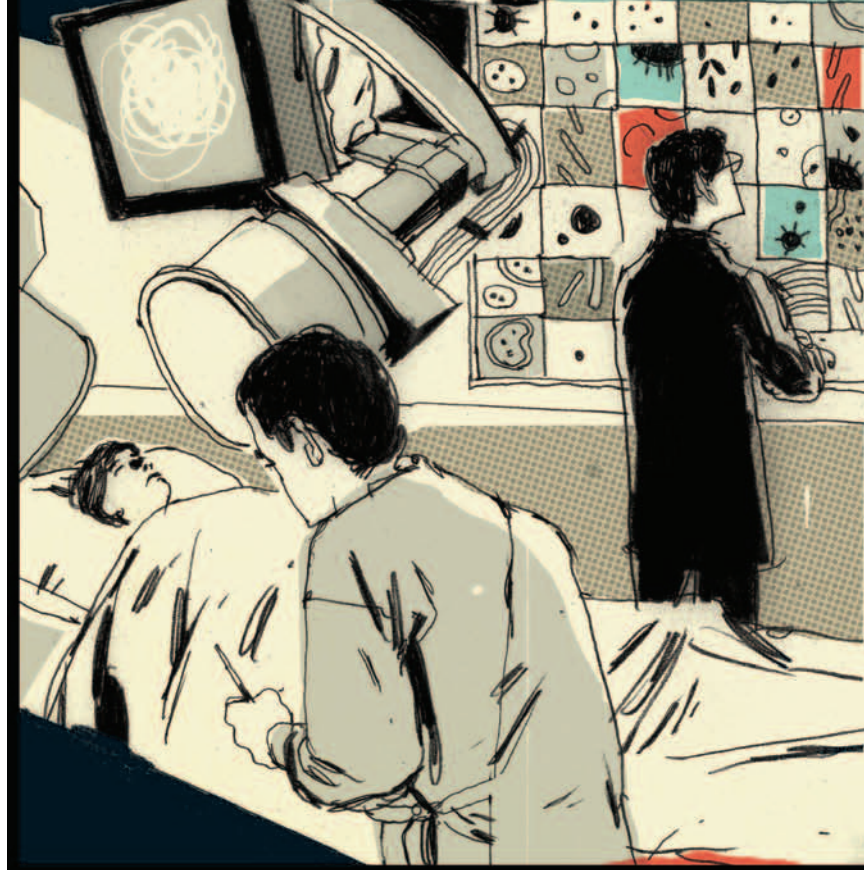
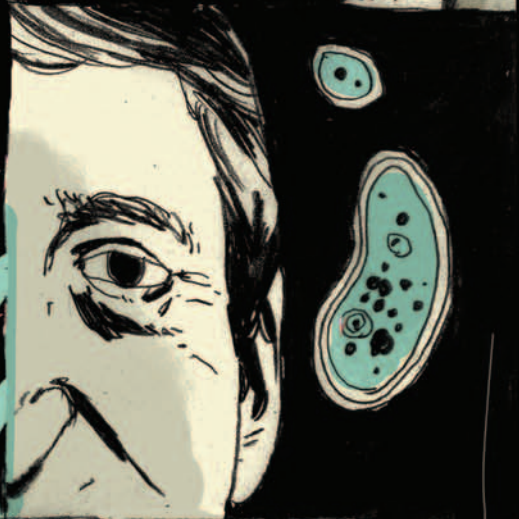


AIDED BY MICROARRAYS, AN INSPIRED PARTNERSHIP COULD FINISH THE JOB STARTED BY PAST PIONEERS.

MODERN-DAY VIRUS HUNTERS

BY STEVE OLSON
ILLUSTRATION BY JOSH COCHRAN





few days before Christmas 2005, a 28-year-old woman drove herself to the emergency room of the hospital at the Stanford University Medical Center. For 10 days, she had been running a fever, coughing, and waking up at night drenched in sweat. An x-ray had revealed fluid in her lungs, and she was taking an antibiotic to cure what doctors thought was a case of pneumonia. But she wasn't getting any better. ¶ When a new x-ray showed that her lungs were even more clogged, she was admitted to the hospital and placed on two more antibiotics. But her condition continued to deteriorate, and on her third day in the hospital she quit breathing and had to be placed on a ventilator. Hospital physicians tested her blood and the fluid in her lungs for infectious agents—they even did a lung biopsy, looking for suspicious bacteria,

fungi, and viruses. “We went through every test we had in the lab,” says Bruce Patterson, the director of virology at the hospital and a professor of pathology and medicine at Stanford University School of Medicine, “but everything came back negative.” ¶ On the woman's eighth day in the hospital, Patterson called Joseph L. DeRisi, an HHMI investigator at the University of California, San Francisco (UCSF). Patterson had heard DeRisi talk about a new device he had built, a microarray spotted with DNA fragments that can detect DNA sequences from all known viruses. Did he think it could determine what was killing the woman? Patterson asked. ¶ Within a few hours, a medical courier was driving a sample of lung fluid up Highway 101 to DeRisi's lab in UCSF's new Mission Bay campus just south of downtown San Francisco. Less than a day later, DeRisi had a diagnosis. The woman was infected with parainfluenza 4, a virus not previously known to cause acute respiratory failure in healthy young adults. ¶ By that time it was too late to give the patient any more medications, but, miraculously, she lived. Her body got the infection under control. Within a few days she was taken off the ventilator, and 2 weeks later she went home.

This woman was lucky, DeRisi and Patterson agree, but relying on luck isn't good enough. “If her doctors had known that it was parainfluenza 4 early on in the hospital stay, treatment decisions would have been different,” DeRisi says. “There would have been no reason to give her all those antibiotics plus an open-lung biopsy, which has some pretty drastic potential outcomes associated with it.” As a result of that case, Patterson set up what he calls a SWAT team to deal with the handful of patients

who show up in Stanford's hospital each year with infections that cannot be identified. When existing tests come up negative, samples can be sent to DeRisi's lab to look for unexpected viral infections.

After all, if doctors don't know what is making a person sick they can't propose the best possible therapies. “It's shooting in the dark,” DeRisi says.

BLAST AHEAD

DeRisi is not a physician, but his work with

UCSF virologist Don Ganem, also an HHMI investigator, has tremendous clinical potential. It's “bench-to-bedside research at its very best,” says UCSF chancellor and Nobel laureate J. Michael Bishop.

After graduating in 1992 from the University of California, Santa Cruz, with an undergraduate degree in biochemistry, DeRisi went to Stanford University to do graduate work in the lab of HHMI investigator Patrick O. Brown. At that time, DNA microarrays were so new that the National Institutes of Health had rejected Brown's initial requests to develop the technology. But one of Brown's favorite phrases is “blast ahead,” and he knew that microarrays were going to be important so he pieced together other funding to work on them.

Analogous to mailboxes in an apartment building, DNA microarrays consist of thousands of distinct DNA fragments attached to

a glass slide. DNA samples that a researcher wants to identify are tagged with fluorescent labels and washed over the slide. Just as letters are sorted into mailboxes, DNA pieces in the sample stick to matching DNA fragments on the slide, allowing the unknown DNA to be identified.

Although DeRisi went to Stanford to study retroviruses, he was soon swept up in the scientific and engineering challenges of microarrays. “The project I was doing required high-density arrays, and no one was going to make them for me,” he says. After building and programming a robot that attached the DNA fragments to the slide using precision-guided metal pen tips, DeRisi and the other members of Brown’s lab used the device to achieve a number of notable firsts. They were the first research group to look at the activity of all genes in yeast simultaneously, for example, and they were the first to use microarrays to explore global gene expression in human cancers.

Brown and DeRisi found they had much in common. DeRisi is a onetime black belt in aiki-jujitsu; Brown is a marathoner. Both are devoted to open-access publishing of scientific articles; Brown was a cofounder of the Public Library of Science, through which DeRisi publishes many of his papers. And both are ardent proponents of making microarrays as inexpensive and accessible as possible. DeRisi has posted instructions on his Web site for how to build a microarrayer, and he has taught highly popular summer courses at Cold Spring Harbor Laboratory in New York and UC Santa Cruz on constructing and using microarrays.

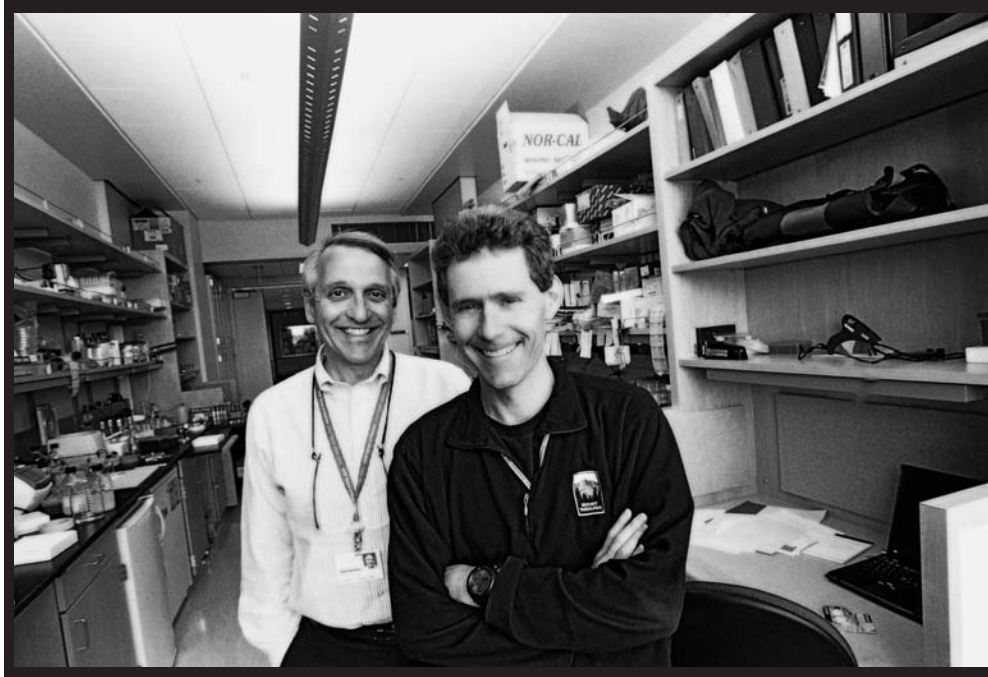
DeRisi’s expertise in molecular biology, bioinformatics, and microarray technology made him a hot prospect, and a postdoctoral fellowship at UCSF was quickly followed by a faculty appointment. DeRisi used his new position to launch a major study on malaria. By tracking the expression of genes of *Plasmodium falciparum*—the parasite that causes the disease in humans—over time, DeRisi and his colleagues have uncovered particular genes that turn on and off in sequence as *P. falciparum* attacks and destroys blood cells. He is working on tests of new antimalarial drugs to see if they interfere with the process.

A FORMIDABLE PARTNERSHIP BEGINS

His malaria studies got DeRisi thinking about other possible medical applications of microarrays, but it took a frustrated postdoc to launch his work on viruses. In Ganem’s lab, postdoctoral fellow David Wang, now an assistant professor at Washington University in St. Louis, had been studying a particular virus-replicating enzyme for 2 years and was making very little progress. Finally, Ganem suggested that Wang try something different by learning about microarrays in DeRisi’s lab. “A couple of months later,” Ganem recalls, “Dave came back to our group meeting and told us that Joe had been talking about using microarrays to detect viruses. Dave asked, ‘Do you think that’s a good idea?’ And I said, ‘It’s a great idea.’”

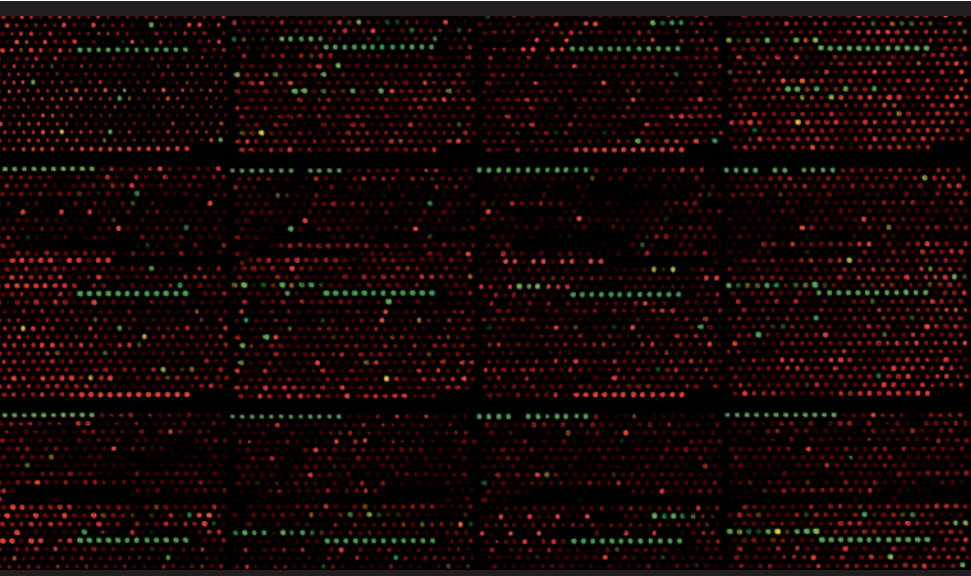
For Ganem, working with DeRisi was a chance to do the kind of science he likes best. After his second year at Harvard Medical School, Ganem took 2 years off to do research with viruses, and after

DON GANEM (LEFT) AND JOE DERISI HAVE HAD A PROMISING FIRST HIT WITH THEIR VIRUS CHIP, UNCOVERING AN INTRIGUING LINK BETWEEN A RETROVIRUS AND PROSTATE CANCER.



“IF HER DOCTORS HAD KNOWN THAT IT WAS PARAINFLUENZA 4 EARLY ON IN THE HOSPITAL STAY, TREATMENT DECISIONS WOULD HAVE BEEN DIFFERENT,” DERISI SAYS. “THERE WOULD HAVE BEEN NO REASON TO GIVE HER ALL THOSE ANTIBIOTICS PLUS AN OPEN-LUNG BIOPSY.”

"I LOVED THE IDEA OF TRYING TO IDENTIFY THE INFECTIOUS CULPRITS OF IMPORTANT DISEASES AND THEN DEVELOPING A COURTROOM STRATEGY TO PROVE THEIR GUILT," SAYS DON GANEM.



THE VIROCHIP V3 ARRAY, DEVELOPED AS A COLLABORATIVE PROJECT OF THE GANEM AND DERISI LABS, CONTAINS REPRESENTATION OF ESSENTIALLY ALL VIRUSES PRESENT IN GENBANK, THE GENETIC SEQUENCE DATABASE OF THE NIH. HERE, THE GREEN SIGNAL SHOWS POSSIBLE POSITIVE HITS, WHILE THE RED SIGNAL INDICATES THE ARRAY WAS CORRECTLY BUILT.

“severe acute respiratory syndrome,” or SARS, the disease soon spread to Vietnam, Hong Kong, and Canada, causing hundreds of deaths. In March 2003, DeRisi and Wang received DNA samples derived from the virus suspected of causing the disease and washed them over their microarray. Within 24 hours, they had identified the virus as a previously unidentified member of the coronavirus family, a conclusion reached at about the same time by another research group using microscopes. A day later, the Centers for Disease Control and Prevention held a news conference to announce the results. In 2004, recognizing his work on both malaria and SARS, the John D. and Catherine T. MacArthur Foundation awarded DeRisi one of its half-million-dollar fellowships—part of which he has used to support malaria studies in Uganda.

completing medical school he did a postdoctoral fellowship in virology. Drawing on those experiences, in the early 1990s Ganem and his UCSF coworkers developed a blood test for infection with herpesvirus 8 that helped establish the virus’s responsibility for Kaposi’s sarcoma, a deadly cancer that is common in people with damaged immune systems. “That was the best moment of my professional life,” says Ganem, “when my knowledge of science and my knowledge of medicine could be used at the same time.”

Wang’s new postdoctoral fellowship in DeRisi’s lab jump-started the virus chip project. Soon they had constructed a DNA microarray designed to identify not only known viruses but also new viruses related to those already known. The microarray contained 10,000 DNA fragments drawn from every human, animal, plant, and microbial virus described in DNA databases at that time. They began testing the microarray against viruses grown in the lab, but then events in the real world intervened.

In late 2002, several hundred people in China came down with a severe form of pneumonia caused by an unknown infectious agent. Dubbed

BUILDING A CASE

“Do you see those little white dots?”

asks Anatoly Urisman, holding a conventional one-inch by three-inch glass microscope slide up to the window. With the sun shining through the slide, square arrays of tiny white dots float into view. “Those are the DNA spots.” He slips another slide into a laser scanner, and a dense grid of green and red circles appears on a computer monitor. “This one didn’t work very well,” he mutters. “Those red spots should be brighter.”

Urisman, an M.D.-Ph.D. student in DeRisi’s lab, is heading up one component of DeRisi and Ganem’s most ambitious project to date. Drawing on his clinical background, Ganem has been obtaining tissue samples from people with respiratory infections, hepatitis, meningitis, rheumatoid arthritis, multiple sclerosis, and a half dozen or so other diseases. DeRisi and his labmates then use the DNA microarray to identify viruses in the tissues. If they find suspicious viruses in patients with a particular illness, those viruses may be clues to the origins or course of the disease.

Earlier this year, the team reported the first of what it hopes will be many hits. At the Cleveland Clinic, oncologist Robert H. Silverman found a subset of prostate cancer patients with a suspicious genetic mutation. The men had a defect in a gene called *ribonuclease L*, which produces a protein that defends against viral infection. With their permission, DeRisi’s lab isolated DNA from the prostate cancer tumors of men with the genetic defect and washed the DNA across its microarrays. The tests produced a clear signal: many of the men

were infected with a retrovirus, called XMRV, related to a virus known to infect mice but not humans.

The nature of the link between XMRV and prostate cancer remains unknown. It's possible that infection with the virus is coincidental, especially since the virus is not of a type known to cause cancer in humans. Or XMRV could cause the cancer indirectly by contributing to inflammatory processes in the prostate. "In my heart of hearts, I don't think this virus is the direct cause of prostate cancer," says Ganem. "A hit on the array isn't the end of a project. It's just the beginning. If we get a connection, we still have to build a case for causation."

Their success with XMRV has demonstrated the potential of Ganem's and DeRisi's approach, but their partnership is not without its challenges. For one thing, they work in different parts of San Francisco. Ganem's lab is in the UCSF hospital overlooking Golden Gate Park and the Pacific Ocean, several miles from DeRisi's Mission Bay lab. The two investigators and their students have become experts at navigating the hilly urban terrain that separates the two campuses.

Organizational and professional issues are more daunting than the logistics. "There are only two outcomes in this research—a home run or a strikeout," Ganem notes. Such riskiness can be hard on graduate students and postdoctoral fellows. If a postdoc is fortunate enough to work on a project that yields a home run, everyone is happy. But a postdoc could spend a couple of years searching for a viral cause of a common disease and come up empty-handed. "I worry about that a lot," says Ganem.

Getting their research funded is another potential problem. A search for the viral causes of common diseases does not fall neatly into established research categories. Fortunately, support from HHMI has allowed the work to go forward. "Every day I thank God that there's an organization that's willing to take those risks," Ganem says.

Despite the difficulties, Ganem and DeRisi have high hopes for their joint project. According to DeRisi, "I don't think either of us on our own would be able to accomplish this project individually, but together we make a really strong team." Other researchers are equally enthusiastic. "There's no question that this technology is going to produce critically important insights," says Herbert W. "Skip" Virgin, who studies the effect of viruses on the immune system at Washington University in St. Louis. "And many more positive results will be forthcoming."

A FANTASTIC SUBVISIBLE WORLD

*In 1926, Paul de Kruif, a University of Michigan bacteriologist turned writer, published a best-selling book called *The Microbe Hunters*. In it, he told stories of "the bold and persistent and curious explorers and fighters of death"—from Anton van Leeuwenhoek to Louis Pasteur to Walter Reed—who discovered and tamed the microbial causes of disease. They "peeped into a fantastic sub-visible world of little things, creatures that had*

lived, had bred, had battled, had died, completely hidden from and unknown to all men from the beginning of time," de Kruif wrote. "Beasts these were of a kind that ravaged and annihilated whole races of men ten million times larger than they were themselves."

Ganem read *Microbe Hunters* while in eighth grade and was profoundly affected by it—an experience not uncommon among physician-scientists of his generation. "For me, it was like a police drama," he says. "I loved the idea of trying to identify the infectious culprits of important diseases and then developing a courtroom strategy to prove their guilt."

The work that Ganem and DeRisi have undertaken is squarely within the tradition documented by de Kruif. They are searching for the elusive microbes that continue to ravage human populations. The search is a difficult one, they acknowledge, because no one knows how many viruses infect humans and what effects those viruses may have. Geneticists have begun to inventory microbes in the environment—for example, J. Craig Venter, one of the people who spearheaded the sequencing of the human genome, reported several years ago on the many previously unknown DNA sequences of microbes he found in samples of water from the Sargasso Sea. But no one has tried to do the same for human beings.

DeRisi and Ganem believe that now is the perfect time to finish the job that past microbe hunters began. As soon as researchers inventory the full range of microbes that infect humans, technologies like DeRisi's microarrays can link those microbes to specific diseases. "Forget the Sargasso Sea," DeRisi says. "Do somebody's nose." ■