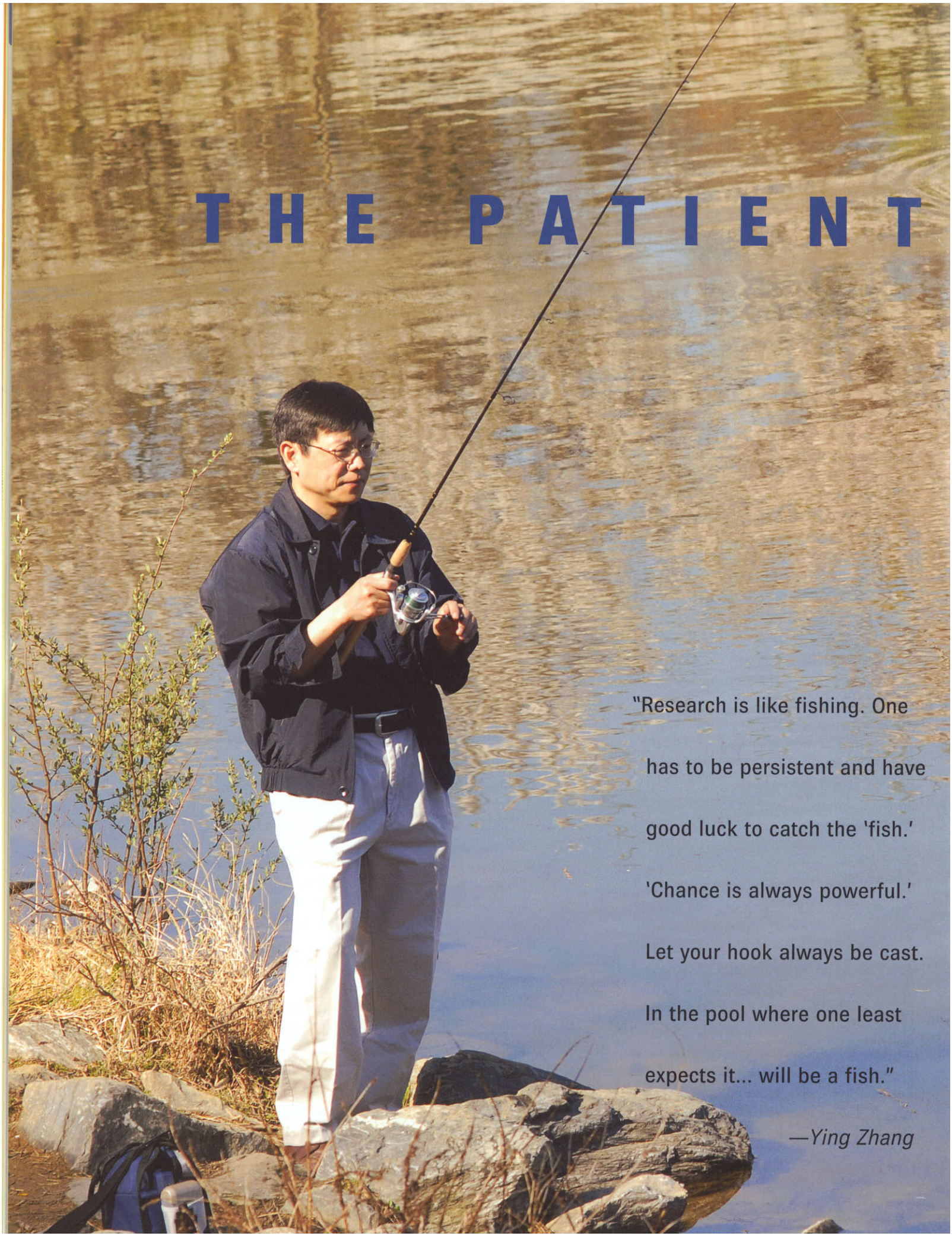


THE PATIENT

"Research is like fishing. One has to be persistent and have good luck to catch the 'fish.' 'Chance is always powerful.' Let your hook always be cast. In the pool where one least expects it... will be a fish."

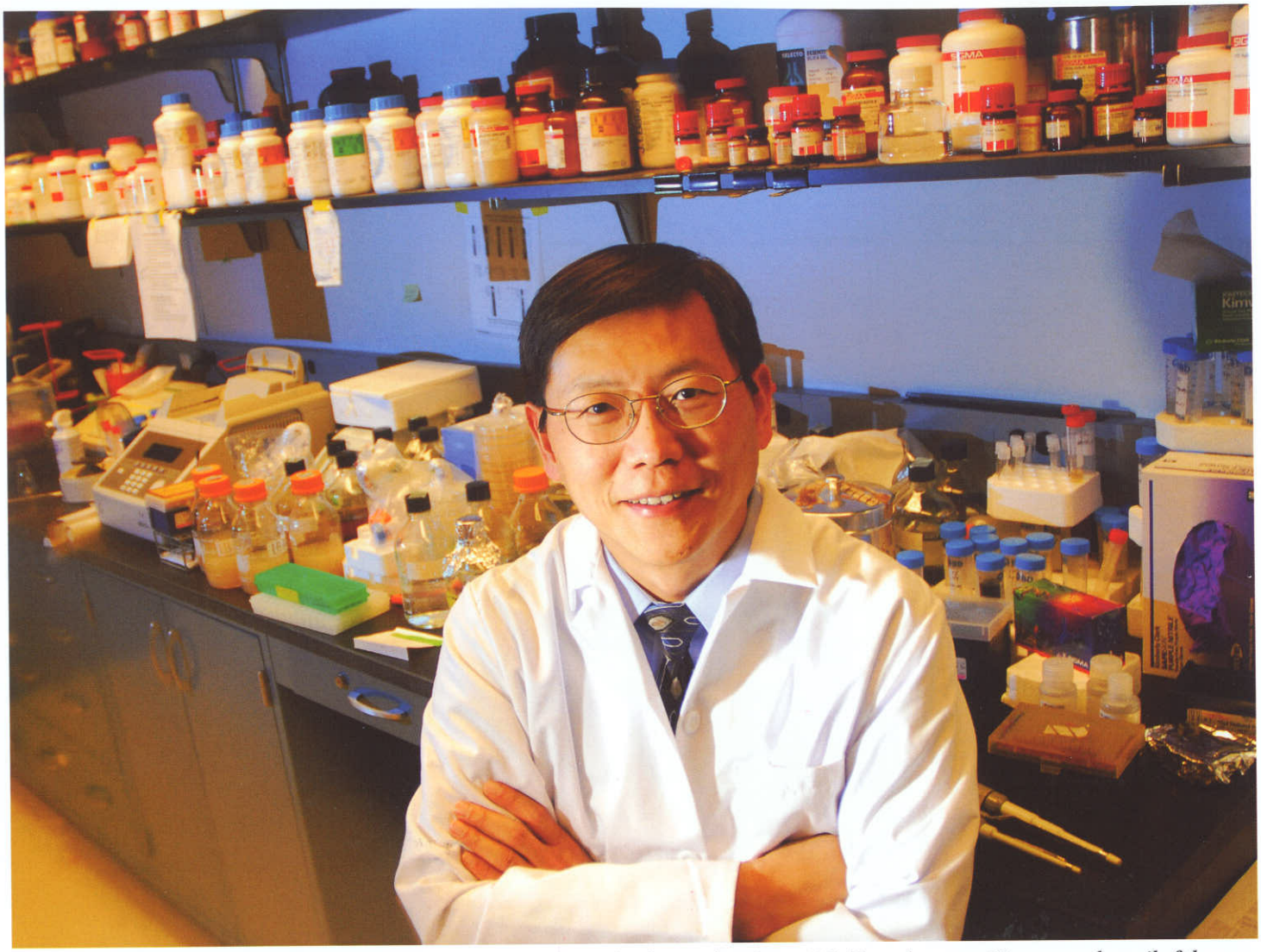
—Ying Zhang

Scientist

For centuries the tenacious bacterium that causes tuberculosis has eluded humanity's attempts to kill it quickly, but one researcher may have landed a better solution.

***Mycobacterium tuberculosis*, meet Ying Zhang.**

by Margaret Guroff
photography by Christopher Myers



A consuming interest: TB researcher Ying Zhang in his lab. A Hopkins faculty member since 1995, Zhang has spent 20 years on the trail of the microorganism that kills 2 million people every year. "These bacteria are smart, I can tell you," he says. "It's not easy to get rid of them."

In 1986, when Ying Zhang started his PhD studies and learned he would be researching tuberculosis, he was baffled. "I thought TB was cured," recalls Zhang, now a professor of Molecular Microbiology and Immunology at the Bloomberg School. "I thought it was not a problem."

This is a common misconception even today, but it is a dangerous one. As Zhang soon found out, the life-threatening disease caused by *Mycobacterium tuberculosis*—a scourge since ancient times—had long defied easy cures. Though its multiplying, rod-shaped bacteria can usually be slaughtered with antibiotics, a sometimes-spherical, dormant form of the bugs cannot. These so-called "persister" bacteria, which evolved to help the species survive stress such as starvation, are thought to slumber for months or even years inside a patient's own immune cells, which themselves nestle within the lungs or other organs. Then the persisters awaken and turn back into replicating rods.

"These bacteria are smart, I can tell you," says Zhang, MD, PhD, sitting at a paper-stacked table in his office. "It's not easy to get rid of them."

What's more, TB is on the march worldwide, fueled by the emergence of drug-resistant strains and by a growing population of vulnerable potential hosts: people with HIV. Tuberculosis, once called "consumption," causes symptoms that include wasting, pallor and coughing up blood. Though six months of an antibiotic cocktail can cure most cases, the disease still kills 2 million people a year. And one-third of the world's population is thought to be infected with TB, which can lie dormant for a lifetime, waiting for an opportunity to strike when the immune system is weakened. (Only about one-tenth of TB infections ever cause symptoms.)

Zhang has spent the past 20 years delicately trying to pick the lock of this diabolical microorganism. A quiet, focused man who earned an MD in his native China and a doctorate in England before joining the Johns

Hopkins faculty in 1995, Zhang is responsible for major advances in understanding how *M. tuberculosis* bacilli attack the body's tissues and how the bacteria become resistant to antibiotics. "Ying is a very, very intriguing guy," says William R. Jacobs, Jr., PhD, of the Howard Hughes Medical Institute, a leading researcher of the genetics of TB. "He's low key, but his contributions are very important."

Now, Zhang's lab is studying what may be the most vexing question of all: how to annihilate stubborn "persister" TB bacteria. In a typical case of full-blown TB, a patient might have 1 billion active bacilli, which succumb to antibiotics within a couple of weeks. But the patient might also have 1,000 to 10,000 persisters that survive the antibiotics. "This is the biggest problem facing tuberculosis control right now," Zhang says.

Humans have contracted tuberculosis for as long as there have been humans, prehistoric skeletal remains show. At times, the disease was thought to be inherited, because frequently more than one family member got it. Some societies even considered TB a sign of vampirism, with a family's first victim accused of returning to slowly drain the life from those who remained. Eventually, TB was recognized as a contagious disease and patients were confined to sanitaria, rural institutions where fresh air and rest sometimes allowed the symptoms to remit.

In 1720, the English physician Benjamin Marten correctly guessed that TB was caused by "wonderfully minute living creatures," but it wasn't until 1943 that researchers developed the first drug that could kill these creatures: streptomycin, an early antibiotic. This one drug alone could not cure the infection, but before long other antibiotics were added to the mix. At last, some TB patients were cured, albeit after as long as two years of daily medication designed to attack the tenacious persisters as they awoke.

In 1954, researchers created a drug called pyrazinamide (PZA), which is still the only medicine that is proven effective against any persister TB bacteria. Even PZA cannot harm persisters that are truly dormant, but it kills the ones that are just beginning to stir. Zhang likens the active and dormant cells of a TB infection to the yin and yang symbols of Chinese philosophy—that is, the opposite and complementary parts of any whole. "Within the persisters there is a small population that want to grow; they want to revert," he says. With the advent of PZA, which doesn't kill actively growing cells, those yearning persisters could be knocked off earlier, and tuberculosis treatment time was cut to six months—the so-called "short course" that is still standard today. "It sounds long," Zhang admits, "but it's the best we have."

The trouble with such a long "short" course is that many patients do not complete it. Their symptoms vanish rapidly at the start of treatment, which lowers their motivation to finish their medicine. But, as with other infections, if TB is not fully wiped out, it can return in mutant forms that are resistant to the drugs it has encountered. This is the origin of multidrug-resistant tuberculosis (MDR-TB), which is impervious to at least two of the drugs in the standard antibiotic cocktail. It is also the cause of a rarer strain, extensively drug-resistant tuberculosis (XDR-TB), which resists some second-line drugs as well. (There are even reports of drug-dependent tuberculosis, in which one of the standard drugs actually fuels the growth of TB bacilli.) Though tuberculosis is vastly more common in developing countries than in the industrialized world, there have been outbreaks of both MDR-TB and XDR-TB within the United States. MDR-TB was present in the U.S. as early as 1970, and, according to the Centers for Disease Control, it has been reported in 43 states plus the District of Columbia. More than 100 cases of MDR-TB are reported in the U.S. each year, and a total of 47 cases of the more lethal XDR-TB have been reported here since 1993.

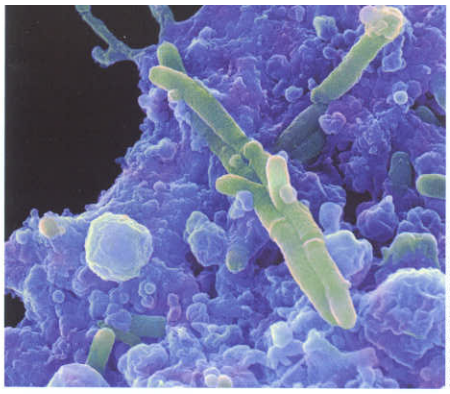
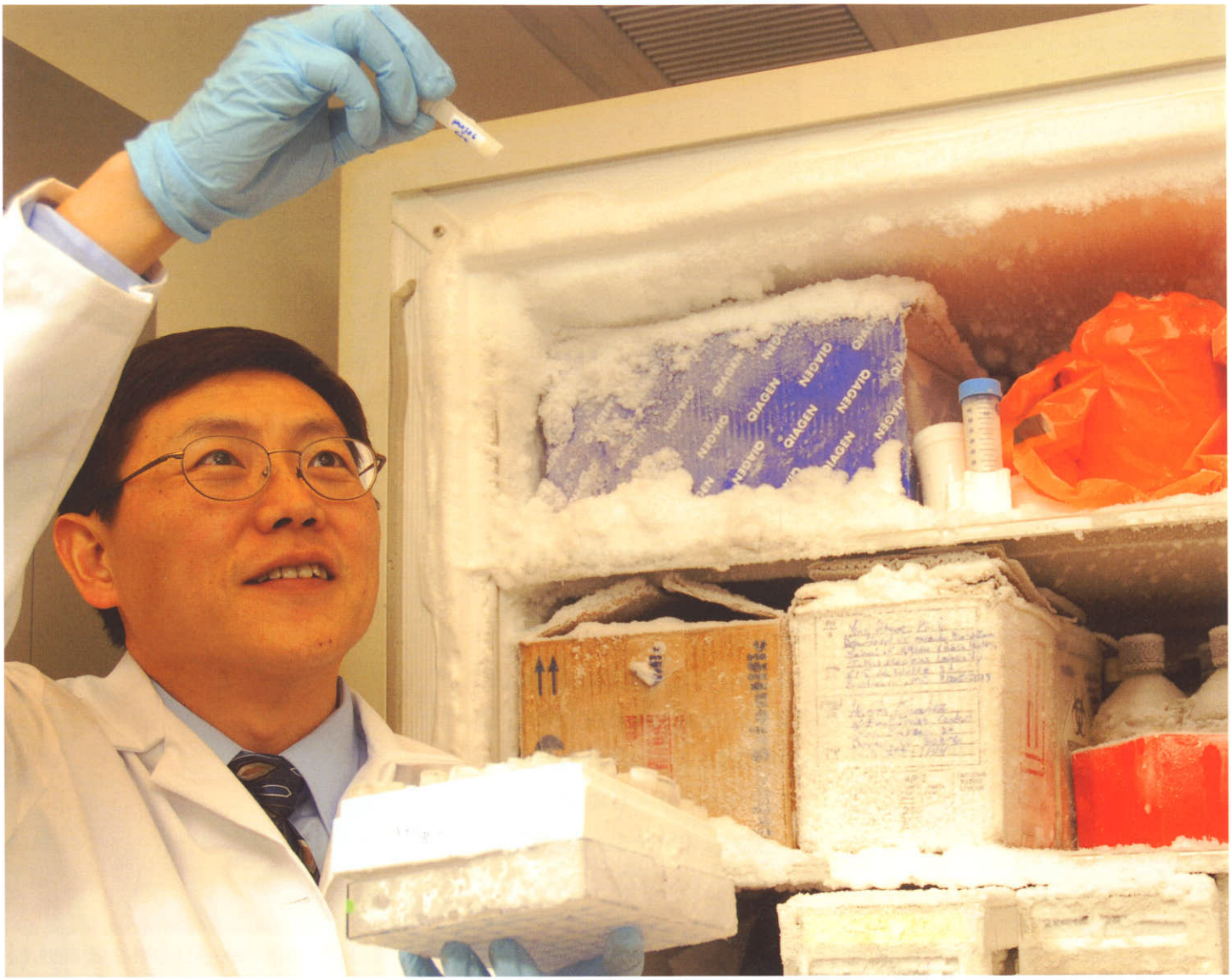


PHOTO RESEARCHERS, INC.

When antibiotic treatments were first discovered, eradicating tuberculosis seemed a real possibility. Then in the 1980s HIV/AIDS emerged, and *M. tuberculosis* went on the offensive, preying on the weakened immune systems of AIDS patients. In 1993, the World Health Organization declared the disease a global health emergency—the only disease ever so dignified.

When antibiotic treatments for tuberculosis were first discovered, it seemed a short time until the disease would be eradicated. But the difficulties caused by the lengthy "short" treatment kept that from happening. And when HIV/AIDS emerged in the 1980s, *M. tuberculosis* went on the offensive, preying on the weakened immune systems of AIDS patients—and spreading, through their coughs, much farther than it could have otherwise. In 1993, the World Health Organization declared tuberculosis a global health emergency—the only disease ever so dignified. The motto of this year's WHO World TB Day on March 24 was a somber reminder of the disease's mobility: "TB anywhere is TB everywhere."

As a young researcher at Hammersmith Hospital in England, Zhang wrote a dissertation that contributed to the current understanding of how the TB bacillus operates in the body. In 1991, he and Douglas Young, PhD, showed how the bug manages to survive while hiding inside white blood cells, the very cells designed to kill invaders. They discovered that TB cells secrete an enzyme called superox-



Bug hunter: Zhang has contributed a pair of key breakthroughs in the race to defeat the drug-resistant forms of the TB bacillus. "Most people would be happy to make one in their career," says one colleague. "And now he's on track to make a third."

ide dismutase, which is, coincidentally, a common ingredient in anti-aging face creams. This antioxidant counteracts a toxic free radical that the body's immune cells use to attack invaders. To many researchers, it didn't make sense for an infectious bacterium to secrete such a soothing substance. "A lot of people were skeptical," recalls Jacobs. "I heard from a lot of people, 'It's just an artifact, it's not real.'" But about four years ago, Jacobs's lab discovered a secretion system in the cells that bore out Zhang's claim of more than a decade earlier. "It really convinced the world that Ying's work was correct," he says.

And in 1992, Zhang and Stewart Cole, PhD, of the Pasteur Institute in Paris, published a stunning paper in *Nature*, identifying a gene that makes the TB bacillus vulnerable to isoniazid (INH), one of the four first-line drugs. TB bacteria without this gene are resistant to the drug. "Ying was able to discover the mechanism of drug resistance for the most important and most widely used drug in TB," says Richard Chaisson, director of the Center for Tuberculosis Research at Johns Hopkins. "That had a really catalytic effect on the field. It was a huge leap forward." Zhang's discovery led to a simple test for INH resistance. Now, rather than blindly treating a patient with first-line drugs while waiting six to eight weeks to culture and test the bacteria for drug susceptibility, doctors can identify many drug-resistant strains—and switch immediately to second-line drugs—using a molecular test that takes just one day.

By 1996, Zhang was at the Bloomberg School, where he and then-post-doc Angelo Scorpio, PhD, found a gene governing resistance to another first-line drug, PZA. Scorpio, now an infectious disease expert at the U.S. Army Medical Research Institute of Infectious Diseases in Fort Detrick, Maryland, recalls Zhang as an exacting boss. "I don't want to say he was 'pushy,'" Scorpio says. "'Energetic' might be a better word for it. It worked out well for me."

Continuing to focus on how drugs affect the TB bacterium, in 2003 Zhang and his colleagues proposed a new model for the way PZA kills persisters. According to this theory, in acidic environments, the active form of the drug disrupts the so-called membrane potential of

TB cells—the ability of the cells to expel positively charged protons as a result of consuming oxygen and sugars for fuel. Membrane potential is measured as an electric charge, Zhang says, and an active, replicating cell might have a potential of 100 or 200 millivolts (mV). A persister, on the other hand, might have a potential of only 50 or 60 mV. Because cell death occurs when the membrane potential approaches zero, a drug that chips away at that potential might hasten or bring about cell death.

Though the question of how PZA works is still not settled, recent findings support Zhang's model. "We predicted that if we have a compound that lowers membrane [potential], it would enhance PZA activity," Zhang says. "We also predicted that weak acids, even ibuprofen, can enhance it." As demonstrated by his lab's research and that of outside scientists, both of these predictions were borne out. And now Zhang's attention has homed in on persistence itself: What allows persister bacteria to sleep through an antibiotic attack? Is there a way to force them to wake up and meet their fate? If such a wake-up call can be found, it could significantly shorten the course of tuberculosis treatment—and thereby significantly reduce the risk of new drug-resistant strains.

One of Zhang's latest discoveries, achieved with his research associate Yongfang Li, PhD, and published online in April by *Antimicrobial Agents and Chemotherapy*, is a gene in another disease-causing microbe, *E. coli*. Zhang describes the gene, called *phoU*, as a "metabolic brake" that enables persisters to form. Disabling this gene causes *E. coli* bacteria to become hyperactive and more susceptible to antibiotics. Zhang postulates that phoU may point to the wake-up call he has been seeking for TB: a switch that can turn persisters back into active cells, so that they lose the ability to hide from treatment. Though the research is preliminary, Zhang and Li have also identified two genes on the TB bacillus that seem to perform the same function.

"Ying has made two major contributions already," says Chaisson, referring to Zhang's discovery of genes governing INH and PZA resistance. "Most people would be happy to make one in their career. And now he's on track to make a third."

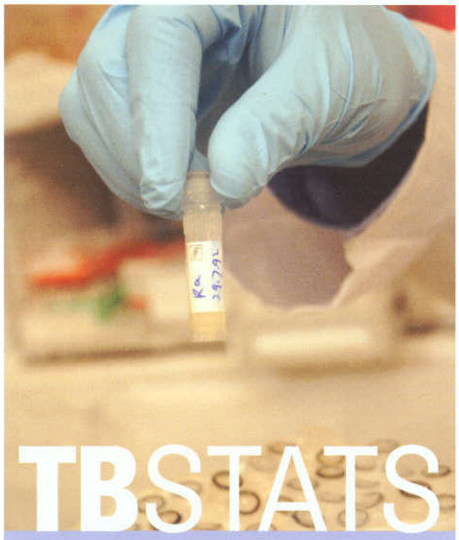
Zhang doesn't downplay the importance of his findings, but he doesn't brag about them, either. He compares science to the study of music. "I used to play violin when I was in college," he says. "To learn, you have to start from the basics. For every single note, there's intonation, there's the bow's speed—you have to master all that to produce beautiful music. It's so amazing; there's so much involved. Research is exactly like that."

Zhang and his wife, Yan Wang, MD, PhD, a researcher at NIH, have lived in the West for decades and are the parents of two school-aged boys whom he describes as "completely American." But Zhang returns frequently to China as a visiting researcher. There he collaborates with clinicians and learns more about how the bacteria function in the real world, not just the test tube. These missions help guide Zhang's research back in Baltimore, including investigation of the still-mysterious drug-dependent strain, which was first observed in Asia. "People should really go to the field," he says. "You find out what really are the problems in the clinic."

After two decades of striving to illuminate the workings of the ancient tuberculosis microbe, Zhang is now moving from basic to applied research. He is beginning the long and unpredictable process of collaborating with drug companies to find therapies that attack persisters at the targets he has identified.

Zhang's lab is also bringing his theories to bear on another disease with tenacious dormant cells that lurk now and attack later: breast cancer. Though most cancer is not understood as an infectious disease, Zhang says that the concept of targeting dormant cells may translate to the so-called cancer stem cells that are responsible for the disease's spread throughout the body. "It's the same principle," he says. "If cancer stem cells are not affected by drugs that kill growing populations, then we need another type of drug."

This interest in cancer doesn't mean, however, that Zhang is leaving tuberculosis behind. He says he is far from finished with the microorganism that launched his career. "I love the bacteria," Zhang admits. "It shows us so much. It shows us the way to persist."



TBSTATS

1: Number of people infected with TB every second

10-15: Number of people that a person with active, untreated TB will infect every year

50: Approximate percentage of those with active TB who will die if the disease is left untreated

200,000: Number of people with HIV/AIDS who die from TB every year. HIV infection is the single greatest contributing factor to the growth of the TB epidemic in Africa since the 1990s.

8.8 million: New TB cases reported worldwide in 2005; 7.4 million of those cases were in Asia and sub-Saharan Africa.

718: Cases per 100,000 people in South Africa

4.6: Cases per 100,000 people in the United States

450,000: Number of multidrug-resistant TB cases detected annually. The highest incidence rate is in China and the republics of the former Soviet Union.

1882: Year of German microbiologist Robert Koch's discovery of the TB bacillus. World TB Day is held annually on March 24, the anniversary of that discovery.

SOURCES: WHO, CDC, American Lung Association