It takes more than a brilliant scientific mind to make a major BREAKTHROUGH

BY ANGELA HERRING, BILL IBELLE, AND JOHN OMBELETS
PHOTOGRAPHY BY BROOKS CANADAY

THE TWISTING ROAD TO A SCIENTIFIC BREAKTHROUGH often includes a period of resistance—and sometimes even open hostility—from those who are still wedded to the mainstream view.

This was certainly the experience of three Northeastern researchers—Kim Lewis, Jonathan Tilly and Lisa Feldman Barrett—all of whom have made major discoveries in their respective fields. For each one, the journey began with something that simply didn't make sense; something that others in the discipline were unable to explain, or chose to ignore.

But a streak of stubbornness, independence, and unquenchable curiosity compelled them to persist, and in doing so, they accomplished what others could not.

What follows is a look at three scientific odysseys, and the roller coaster of frustration and success that, in the end, led to discovery.

SEEK AND DESTROY

By Bill Ibelle

Kim Lewis' 13-year scientific journey began with a paradox—and climaxed with a potential cure for MRSA, the so-called "superbug" that kills more than 10,000 Americans each year because of its ability to elude conventional antibiotics.

If his discovery makes it to market, it will provide a cure for hundreds of thousands of potentially fatal infections in the heart, lungs, and bones. In addition, Lewis is applying his breakthrough to parallel research he's conducting on Lyme disease and antibiotic-resistant tuberculosis, as well as the development of new antibiotics.

As the director of Northeastern's Antimicrobial Discovery Center, Lewis has dedicated his career to tracking and killing the elusive "persister cell" that makes chronic infections nearly impossible to treat. You could think of the cells as the Bin Laden of infectious disease, hiding until the coast is clear, and then reemerging to wreak havoc of catastrophic proportions.

ANTIBIOTIC-RESISTANT INFECTIONS

The paradox Lewis encountered more than a decade ago was this: Traditional antibiotics killed the new superbugs in a petri dish, yet couldn't touch them in humans. It didn't make sense. Furthermore, the paradox had become a medical crisis as the number of superbug infections rose dramatically. MRSA *(Methicillin-resistant Staphylococcus aureus)* infections, which more than doubled from 2003 to 2008, cause more than 18,000 deaths per year, according to the Centers for Disease Control and Prevention.

The explanation, widely accepted in the medical community, was that the antibiotics couldn't reach the superbugs because they lived within the protective fortress of biofilms—a sort of microbial slime that forms around surfaces within the body. This biofilm, according to conventional wisdom, had unknown properties that made infections impervious to antibiotics.

"These pathogens seemed invincible," says Lewis, a University Distinguished Professor of Biology. "The standard drugs were not working. It looked like a problem without a solution."

Which is exactly what attracted Lewis.

"It was the ultimate tough problem," he says. "I had always been attracted to puzzles and paradoxes."

The superbug seemed like the perfect focus—complex, extremely important, and enigmatic to scientists.

"Instead of solving the problem, the scientific community was sweeping it under the rug," says Lewis.

Convinced that the biofilm "wasn't the real culprit," he staged a series of experiments to test the theory and quickly demonstrated that conventional antibiotics did, in fact, kill most of the bacteria within the biofilm. So why did the infections keep coming back?

"The reason appeared to be connected to a small subpopulation of cells that seemed invincible," he says.

But if this was true, why hadn't others reached this same conclusion?

So Lewis printed out every study he could find on biofilms, divided the foot-high stack with his postdoc, and found four papers that described a similar experiment. Every one of them ignored the subpopulation.

Lewis, who has uncanny recall, remembered a paper he had once read that was published by an Irish microbiologist in 1944. The author had been trying to kill staph infections with penicillin and was frustrated that a small subset of cells survived no matter how much penicillin he used. The author named his discovery "persister cells" and, to Lewis, they sounded exactly like what he was up against.

"The only purpose of persisters is to survive," he says. "They don't do anything else."

As soon as antibiotic treatments are applied, the persister cells go dormant. Since antibiotics only attack active cells, the persisters survive, and when the antibiotic treat-

Kim Lewis, University Distinguished Professor of Biology

ment is completed, they come out of their sporelike state and the infection resumes its growth.

"So we had met our ultimate adversary," says Lewis. "We had found the culprit that had been overlooked. I was very excited."

RESEARCH-RESISTANT SCIENTISTS

As it turned out, the scientific community was far from excited about an iconoclastic theory that would turn decades of scientific research on its head.

Lewis sent his paper to the prestigious journal *Science*. They weren't interested. So he soldiered on, publishing his paper in another journal and eagerly awaiting the reaction from his fellow scientists.

Silence.

"It was completely ignored," he says. "It was like it never happened."

So a year later, in 2001, he published a similar paper with a more confrontational title that he was sure the scientific community could not ignore. The paper, he recalls, "caused quite a commotion." When a graduate student who worked in Lewis' lab asked about it, he responded, "You have just helped to close an important field of science."

A few months later, Lewis was slated to chair a session at the annual conference of the American Society for Microbiology. His session was the day after one that focused on biofilms.

"So I went to that session and waited for my opportunity," he recalls. "During the Q&A, I said, 'There is nothing unique about the biofilm's resistance to antibiotics.'" He told the audience he had the research to prove it and invited them to attend his presentation the next day.

"They came and there was a vigorous discussion. I told them, 'Look, this is a very simple experiment,' and challenged them to reproduce it and see if they got the same results."

Apparently they did, because quietly over the next several years, the field of biofilm resistance to antibiotics disappeared and Lewis received substantial funding in government grants for his research.

In spite of his years in the scientific wilderness, Lewis says he never doubted that he would win the battle against persister cells and the resistance of the scientific community.

"Science does not treat wimps kindly," he says. "You must have a thick skin and an enormous tolerance for defeat."

Lewis was no longer in scientific exile. He had identified the enemy and had a well-financed lab at Northeastern, with a team of dedicated graduate students and postdocs to help him with his research.

The first step was to isolate the persister cells—a difficult task because they were "slippery" and no one knew much about them. But by 2006, he had succeeded in isolating some and published several papers on this. This time, his work was widely accepted.

Lewis was on a roll and says he "naively" thought the next step would be relatively straightforward. All he had to do was determine the mechanism used by persister cells to shut down, and then find a compound to interrupt that process.

"This is one of the dangers of science," he says. "It's like a chess puzzle that seduces you with a solution that is simple, but wrong."

What Lewis soon discovered is that persister cells don't have a mechanism that shuts them down; they have at least 10.

"It was like a Hydra head—cut off one and two more grow back," he says. "It looked like we had hit a dead end. The persister cells were invincible. There was nothing that could touch them."

THE SCIENTIST'S ENEMY

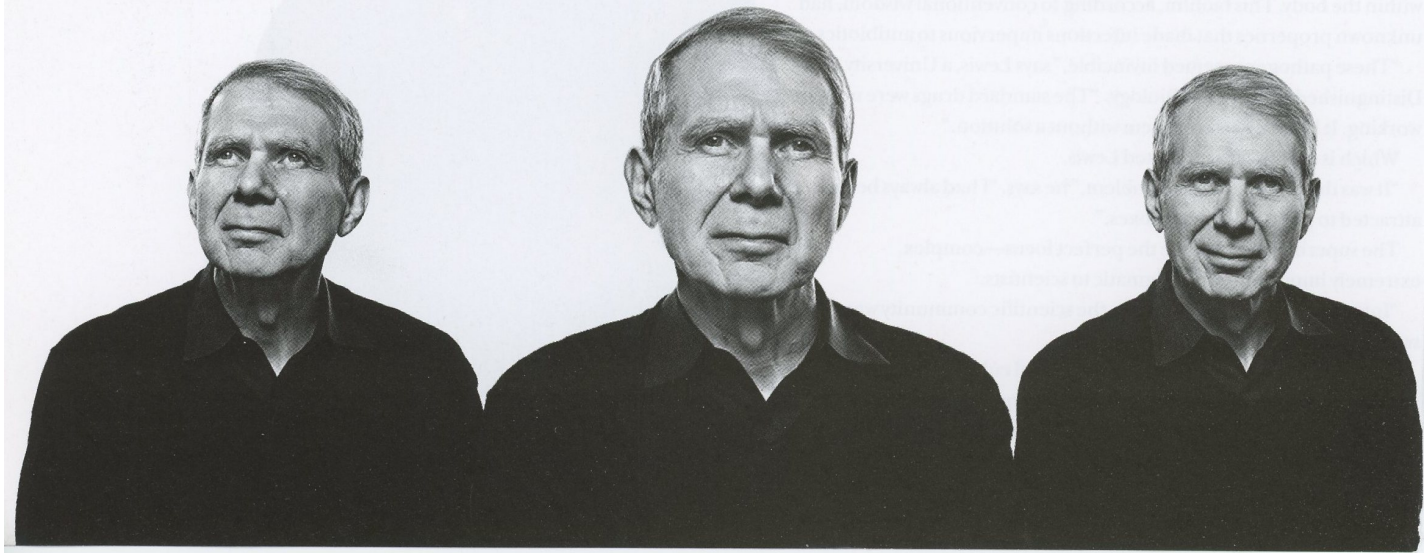
It appeared that Lewis had just completed a decade-long wild goose chase. There was only one thing to do.

"This is part of what I teach my students—how to shut down your common sense," he says. "You have to start looking for a perfect solution and ignore whether it's realistic. That mindset helps you battle your 'common sense,' which is what prevents you from inventing new things."

Lewis started to think about the problem differently. What would an ideal solution look like? First, he would need something to activate an important process in the dormant cell. Next, he would need a compound that would corrupt that process, and in doing so, kill the cell.

"That's the solution—but of course, it sounds far-fetched," he admits.

So Lewis started looking for such a magical compound. He recalled a compound discovered and discarded by Eli Lilly in 1985.



"They didn't even publish a paper about it," says Lewis. "I read about it in a patent."

Lewis was intrigued because the compound, ADEP, takes a different route to cell destruction. It activates an enzyme (ClpP) within the cell that causes the cell to cannibalize itself. This looked promising.

"SCIENCE DOES NOT TREAT WIMPS KINDLY. YOU MUST HAVE A THICK SKIN AND AN ENORMOUS TOLERANCE FOR DEFEAT."
–BIOLOGY PROFESSOR KIM LEWIS

He investigated further and he found that ADEP was picked up by a second pharmaceutical company, Bayer, but soon dropped because cells quickly developed ADEP-resistant strains. Experiments performed by other researchers also indicated ADEP only worked on rapidly growing cells. If this was true, he had hit another dead end.

But Lewis didn't buy it.

"I knew that nature doesn't make junk—and this compound sounded like junk. It only hits growing cells, it hits an unessential target, and resistance develops quickly. Why would nature bother making something like that?"

Lewis looked into these studies and noticed the hole he was looking for—exposure times. They were too short. So Lewis and his postdoc, Brian Conlon, reran the experiments with a 24-hour exposure time and it worked. ADEP killed persisters.

"We were revved up," he says. "ADEP is now going to activate ClpP, chop up every protein in the cell, and force the cell to commit suicide by self-digestion. They were committing suicide. It's a beautiful mechanism."

But there was still the issue of the ADEP-resistant mutants Bayer had identified. Again, Lewis had a theory.

He paired ADEP with a conventional antibiotic and, just as he had hoped, the antibiotics that couldn't touch dormant persister cells could easily kill the mutants.

"It turns out that these ADEP-resistant mutants are wimpy," he says. So as long as you hit them hard with an antibiotic right away, they never get the chance to propagate.

Tests in Lewis' lab killed 100 percent of the pathogens in test tubes and in mice. Then Lewis worked with Steve Leonard, an assistant professor at Northeastern who specializes in pharmacology, to test the combination in a model emulating human infections. The result?

"Complete sterilization."

The results were published in *Nature* in November 2013. Lewis had solved the puzzle.

Bill Ibelle is executive editor.

THE DIFFUSION CHAMBER

AT the same time Kim Lewis was decoding the mysteries of persister cells, he teamed up with Northeastern microbiology professor Slava Epstein to search for new antibiotics.

In 2001, the two men set out to solve a puzzle that has confounded scientists for more than a century: Why are scientists unable to grow 99 percent of the world's microbes in a petri dish?

The question is far from academic. It means that scientists can use only 1 percent of the material on the planet that might lead to new medicines. With this severe limitation on raw material, it's no surprise that the development of new antibiotics has slowed to a trickle in the past four decades.

For Lewis, it was another intriguing paradox: Why do these microbes flourish in the wild, but won't grow in the most nutrient-rich laboratory medium?

"This seemed like a fascinating puzzle to solve," says Lewis. "Slava and I did not focus on developing a better medium. A hundred years of failure suggested that that was not a good way to go."

Instead, they looked for a way to simulate the wild environment while maintaining the controlled setting of the petri dish.

The result was the invention of the Cultursys diffusion chamber, which Lewis describes as "a relatively simple contraption" that makes it possible to grow microbe colonies in their natural environment while maintaining the control of a lab. It consists of two semipermeable membranes (think coffee filters) about the size of a beer coaster separated by an O-ring. A sample of soil containing the microbes is placed in the doughnut hole of the O-ring, and the membranes that seal the top and bottom allow chemicals (but not the cells themselves) to flow back and forth between the artificial and natural environments.

"The bacteria perceive it as their natural environment," says Lewis. "They don't know we tricked them, and as a result, everything grows."

The next task was to identify those growth factors and begin testing the tens of thousands of previously ungrowable microbes to see which ones held the key to the next drugs.

In 2006, Northeastern received a patent for the Cultursys diffusion chamber while Lewis and Epstein formed a company, NovoBiotic Pharmaceuticals of Cambridge, Mass., to develop new antibiotics. So far, 25 have been isolated. Although most appear to be too toxic for human use, two look promising:

- Novo 25, which could replace penicillin, is in the advanced stages of development.
- Lassomycin, a potential treatment for antibiotic-resistant tuberculosis, is still in the test tube stage of development.