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Expertise and Partnerships Combine to Restart the Tuberculosis Drug Pipeline

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“The tuberculosis bacterium is an extremely difficult bug to work with,” says Tanya Parish, Ph.D., a microbiologist specializing in tuberculosis research.

From a medical point of view, that’s something a lot of people would say – for first-line cases of tuberculosis (TB), treatment involves a combination of four drugs for six months. Therapy for more serious cases – called multi-drug-resistant TB (MDR-TB) – can take as long as 24 months, require dozens of drugs and is only successful in 60 percent of patients. The World Health Organization (WHO) estimates that some nine million people develop active TB each year worldwide and about 1.7 million people die from the disease.

But Parish, vice president for drug discovery at the nonprofit Infectious Disease Research Institute (IDRI) in Seattle, is speaking not as a clinician but as a scientist engaged in an intensive effort to find molecular compounds – new drugs – that can kill the TB bacterium, *Mycobacterium tuberculosis (M.tb.)*. “This microbe is very clever,” she says. “Normally when a body’s immune system detects a pathogen invading its tissues, the macrophages, or white blood cells, engulf the organism and kill it. But often, the TB bacteria are able to stop that happening and can survive and even multiply inside immune cells.”

“These bacteria have a thick, waxy coat that stops a lot of drugs from getting inside and making treatment very difficult. They can live in the body in an almost inactive state and hang around for a long time before finally growing and causing the disease we know as TB.”

A Worldwide Effort Along Many Paths

In the big picture, the worldwide effort against TB is a multilayered endeavor that involves consortiums of government agencies, academic institutions, private research institutes and nonprofit foundations following many different paths. Missions are diverse, starting with projects like IDRI’s to discover new drug candidates. Others may further develop identified candidates, or focus on animal testing or human clinical trials, often involving combinations of drugs.

The drug discovery challenge at the front end of the spectrum is the focus of the Lilly TB Drug Discovery Initiative (LTBDDI), a worldwide consortium established in 2007 by Eli Lilly and Co., IDRI and the National Institute of Allergy and Infectious Diseases at the National Institutes of Health. After its formation, the Lilly Initiative quickly gained momentum and new collaborative partners. The founding members were joined by Academia Sinica in Taiwan and a large number of collaborators, including Jubilant Biosys in India, Merck and Co., Weill Cornell Medical College in New York, the Microbial Chemistry and Research Foundation in Japan, Summit PLC in the United Kingdom and the Seattle Biomedical Research Institute.

A Worldwide Affliction Intensified by Poverty

WHO estimates that one-third of the world’s population is infected with the TB bacterium. Most infected persons are protected by healthy immune systems; they carry the infection as inactive “latent TB.” Weakened immune systems make people with latent infections vulnerable to developing “active” cases. The disease is present in every country in the world but it’s particularly prevalent in countries like India and China and in sub-Saharan Africa, where poverty and lack of health resources intensify its reach.

Most new cases of active tuberculosis are considered “drug-sensitive TB” – likely to be responsive to the basic four-drug regimen. Four-drug combinations are needed to overcome the bacterium’s ability to develop antibiotic resistance. When treated appropriately, the cure rate is around 95 percent.

But adherence can be spotty, especially in resource-poor settings, and poor compliance with that regimen can lead to development of multidrug-resistant TB (MDR-TB). This is the serious form of TB that involves complex treatments and, too often, poor outcomes. It’s estimated that some 500,000 people develop MDR-TB annually. In recent years, cases of “extensively” and even “totally” drug-resistant TB” have been reported in South Africa, Iran and India. A grave complication is that, since TB weakens its patients’ immune systems, co-infections with the HIV virus have become a serious problem in many parts of the world.

A Changed Policy and a Crisis in Supply

“Lilly was manufacturing two of the second-line drugs most commonly used to treat TB – capreomycin and cycloserine – when the World Health Organization changed its policy to advocate treatment of multi-drug-resistant TB in 1998,” says Gail Cassell, Ph.D., then vice president for scientific affairs and distinguished research scholar at Lilly, now vice president for TB drug development at IDRI. “Previously, it wasn’t considered cost-efficient to treat MDR-TB patients.

“It became clear to us at Lilly that we wouldn’t be able to supply as much of the drugs as would be needed. We knew we had to look for partners.” In 2003, the company committed $70 million to launch a broad collaborative effort called the Lilly MDR-TB Partnership (The drug discovery initiative would follow in 2007). One goal of the broader effort was to make the technology for its two drugs available to other manufacturers. Today, with 18 partners on five continents, the consortium seeks to train health care

workers, support research and strengthen awareness of MDR-TB on the global health agenda. Lilly put an additional $50 million into the partnership in 2007 and yet another $30 million in 2011.

The drug discovery initiative – the LTBDDI – was created to confront the lack of potential drug-candidates for treating TB – there hasn't been a new TB drug approved for use in more than 40 years. In 2006, Cassell and her Lilly colleagues encountered an intriguing possibility in the midst of discussions about Lilly's acquisition of a Seattle biotechnology company called ICOS Corporation. ICOS had personnel, equipment and capabilities that Lilly wouldn't be utilizing there.

"We inquired as to whether there were chemists who would be interested in working on neglected tropical diseases, and several were," Cassell notes. "We began looking for organizations within the Seattle area that would be interested in housing an infectious disease unit. It became clear that IDRI would be an excellent fit." Subsequently, ICOS chemists Ed Kesicki, Ph.D., and Joshua Odingo, Ph.D., moved to IDRI.

Restarting the Drug Pipeline

"In developed countries we think of TB as a disease of the past but it is endemic in many underdeveloped regions of the world," Cassell says . "With the rise of increasingly drug-resistant strains, without steps to create effective treatments it will become a significant disease. We saw an urgent need to restart the TB drug pipeline."

The LTBDDI was funded by a $15 million Lilly grant, including $9 million in kind and $6 million in cash to seed the organization. In line with the initiative's nonprofit status, it is based at the non-profit IDRI, which serves as the initiative's principal coordinator. In 2008, IDRI recruited another ex-ICOS employee, Allen Casey, to set up a new high-throughput laboratory using equipment donated as part of the Lilly Initiative – high-throughput is a term for screening large numbers of compounds for their characteristics using mechanical or "robotic" devices.

Finding a New Approach

One of the projects that forms part of the drug discovery work at IDRI centers on an assay, or test, developed at Weill Cornell Medical College. "The standard way to test a compound's ability to defeat a pathogen," notes Carl Nathan, M.D., chairman of Weill Cornell's Department of Microbiology and Immunology, "is to grow the pathogen in a test tube and then find something that prevents this growth. You would find something that prevents growth and is safe for humans and give it to people. That worked pretty well for a long time, but it's not working anymore."

Two of Nathan's students evolved a new approach. Omar Vandal, now at the Bill & Melinda Gates Foundation, discovered that acidity does not automatically kill TB. The question was, why not? Vandal scrutinized 10,000 genes one at a time – and developed a genetic screening technique for the bacterium. Another graduate student, Crystal Darby, perceived that Vandal's approach could be used for an investigative chemical screen, essentially measuring the interaction between the pH inside the bacterium cell and comparing it to the pH outside the cell.

M.tb. thrives in a physiological environment with a relatively neutral pH of 7.4 but unlike other pathogens, it can also survive the acidified environment that the host's immune system throws at it. The bacterium adapts to the acidic onslaught by flipping genetic switches. The idea is that identifying chemical compounds that interfere with these switches should prevent the bacterium's survival. A screen of 2,000 compounds was enough to demonstrate the value of Darby's technique.

"Crystal submitted the project to LTBDDI and it was selected," Nathan notes. "She went out to IDRI and showed them how she did the assay and they converted it to their own technology to speed up the process."

He adds: "This is science at its best – a team of colleagues who work together to advance knowledge and capabilities."

The Libraries

It's a saying in TB research that successful drug discovery requires screening a million compounds to find 10 possible candidates – and then it's possible that those 10 won't yield even one new drug. With Darby's assay and IDRI's high throughput screening capabilities in place, the other piece of the puzzle – or the other one million pieces – was to acquire a collection of prospective compounds to screen.

And one aspect of the initiative stands out as strikingly innovative for pharmaceutical research – the commitment of pharmaceutical manufacturers like Lilly to provide other initiative members with access to their vast libraries of proprietary compounds.

"It's every research chemist's standing operating procedure to contribute to a library of compounds for future use," says Philip Hipskind, Ph.D., senior research fellow and group leader at Lilly's research laboratories in Indianapolis. "Any pharmaceutical manufacturer is going to have a corporate-compound collection, samples saved in solution or powder forms for possible future research. For every compound a scientist makes, he or she might take 10 to 15 milligrams for the project at hand and save 50 milligrams for a library."

Lilly's library goes back more than 100 years and contains some seven million compounds – nearly all of our products and current research projects area legacy of this collection, Hipskind notes. Lilly made likely compounds available in two libraries. The "Strategic Screening Paradigm" collection included some 28,500 molecules previously identified as potentially active against human targets. The "Diversity" library contained more than 63,000 molecules that weren't identified as active but that share promising characteristics.

The Screening

IDRI is one the few places in the world in which compounds can be tested against live M.tb. bacteria owing to its specialized equipment and facilities. Whereas a decade ago a scientist could only screen a few hundred compounds in a week for activity against MTB, IDRI's high throughput robotics system makes it possible for them to test 10,000 compounds in a few days. The system consists of a carousel with vertical banks of plates, liquid handlers to add reagents, incubators and robotic arms that move the plates though the automated stations.

"Each plate has 384 individual wells, so we can test almost 400 compounds simultaneously," Casey notes. "A very small sample of each compound, less than the size of a small drop out of an eyedropper, is placed in each well with a small amount of buffer – a fluid that keeps the pH constant at 4.5." The plate is transported to IDRI's Biosafety Level 3 (BSL3) facility, where the screening is performed. In the BSL3's sterile environment, a minute sample containing thousands of bacteria is added to each well and allowed to incubate for two days. Analysis using light waves determines whether each compound has been effective. The reams of data produced are analyzed with the help of a laboratory information management system.

"The goal is to overwhelm the bacterium with a compound that interferes with its ability to withstand acidification," Casey says. "Each compound introduced works differently. It induces a different physiological response. In screening 100,000 compounds, we expect to get perhaps a couple of hundred that are promising."

Next: Toxicity, Pharmacokinetics

That's not the end, however. Those hundreds of "leads" have to be tested in mammalian cell cultures for toxicity, weeding out compounds that would harm the patients they're supposed to save, then into chemistry analysis to predict how each candidate-compound would work.

"TB is quite complicated, existing in different states," says Joshua Odingo, director of chemistry at IDRI. "The issue is not just to prove that something can kill the TB bacterium but to gauge how effective it would be in a person in terms of absorption, distribution, metabolism, and excretion. This in vitro work helps us predict what's likely to happen with a compound in the body." By the end, the number of candidates will go from hundreds down to dozens.

Once those are identified, the IDRI scientists send results to their colleagues at Lilly, who utilize computational capabilities to identify other, similar compounds in their library to send for more screening. The cycle is repeated several times.

"This saves time," Casey says. "Once we have some promising candidates we can focus on other compounds with patterns of similarity. Many times, you pull up a compound that's even better." The entire process takes about three years. By early 2012, the IDRI and Lilly team had finished the initial screenings and were in process of evaluating toxicity in some 500 compounds. Eventually, the work will enter the pharmacokinetics phase, a process that will be performed by IDRI, Lilly and other collaborators.

"Pharmacokinetics and efficacy testing in an infection model is the final stage of drug discovery," Parish notes. "You need to test the compound in an infection model – that is, animal tests. What works in a test tube doesn't always work in an infected body." Eventually, any truly promising compounds may be studied, tweaked and advanced to human clinical trials.

A Learning Curve in Science and Collaboration

Bringing together so many diverse partners in a common endeavor hasn't been without its challenges. For one thing, academics have an instinct for advancing knowledge and corporate scientists layer an additional concern for proprietary intellectual property. "Everyone agrees on the importance of publishing results," says Nathan, "but not necessarily how detailed the information should be. Everybody has good intentions and when we encounter obstacles we work around them."

Adds Erik Iverson, IDRI's executive vice president for business development and external affairs: "These collaborations require an effort to understand the perspectives of everyone involved and their expectations. We addressed these issues with a memorandum of understanding among all the parties. To protect its intellectual property, for example, Lilly provided blinded compounds for screening. A shared goal, the commitment to accommodate each other – and flexible legal agreements – have gone a long way in assuring success."

"It's been interesting to see how this public-private partnership has blossomed," Lilly's Hipskind says. "When it started, a lot of our scientists didn't know what to make of it. Now they're fully on board with this project which focuses on a major medical need. The initiative started with just Lilly, IDRI and NIH. Then it snowballed. Other organizations joined. Researchers from many academic institutions have come to us to say 'We have compounds that are active. Can we offer them?'

"A good two-thirds of what's going on in this initiative is 'open innovation,' he adds. "And it is truly what makes this project really interesting."

By Ralph Fuller