HIV and Tuberculosis: A Lethal Convergence

By Jon Cohen

In 2005, researchers in South Africa came face to face with one of their deadliest fears – the emergence of a strain of tuberculosis that could dodge every drug thrown at it.

More alarming still, the outbreak surfaced in KwaZulu-Natal province, an epicenter of the AIDS pandemic that had hospitals filled with people whose immune systems were already decimated by HIV. As the shocking news made headlines worldwide, Tugela Ferry, South Africa became ground zero for the lethal convergence of HIV and a new threat, extensively drug-resistant tuberculosis (XDR-TB).

The outbreak helped convince the Howard Hughes Medical Institute to work with the University of KwaZulu-Natal to create the KwaZulu-Natal Research Institute for TB-HIV (K-RITH) in Durban, which opened in 2009. This October, K-RITH will move into a new, state-of-the-art building. “This is a tremendous opportunity to bring basic science right to the epicenter of HIV and TB,” says William R. Bishai, a leading TB researcher who heads K-RITH and also maintains a lab at Johns Hopkins University School of Medicine in Baltimore, Maryland.

Since the 1940s, antibiotics have cured TB. But the bacterium that causes the disease, *Mycobacterium tuberculosis*, keeps evolving to dodge most drugs thrown at it. In the late 1980s, multidrug-resistant TB (MDR-TB), which requires a more expensive and toxic second-line treatment, became a major health problem. By early 2006, about 10 percent of MDR-TB in the KwaZulu-Natal region outwitted all first- and most second-line treatments, leading to XDR-TB.

South Africa has more HIV-infected people than any country in the world and one of the highest rates of TB per capita. Of the estimated 5.7 million HIV-infected people in the country in 2009, 250,000 each year also develop active TB, accounting for about one-third of cases of HIV/TB co-infection in the world. It’s more dire still in KwaZulu-Natal, the province that includes Tugela Ferry, where about 17 percent of the general population is infected with HIV, and a staggering 80 percent of adults who have TB are also HIV-infected.

The outbreak of XDR-TB in Tugela Ferry in 2005 involved 53 patients at Church of Scotland Hospital. All but one quickly died. On average, these patients only lived 16 days after their sputum samples were collected for testing — too short a time to even receive the results of the cumbersome test used to diagnose XDR-TB.

Of the 44 XDR-TB patients in the Tugela Ferry outbreak who were also tested for HIV, all were positive. “They were dying like flies,” says Salim S. Abdool Karim, an epidemiologist at the Nelson R. Mandela School of Medicine at the University of KwaZulu-Natal (UKZN) in nearby Durban. “I initially thought this was an isolated outbreak and would burn itself out. I was proven wrong as several hospitals started reporting cases of XDR-TB.” To date, South African researchers have uncovered XDR-TB cases in every province, recording 573 cases in 2008 alone.

“Tugela Ferry was the cataclysmic event that brought this problem to the world’s attention,” says Richard E. Chaisson, an HIV/AIDS and TB specialist at Johns Hopkins. “If it weren’t for that, no one would really be aware of it.”
Better infection control practices have helped curb XDR-TB in Tugela Ferry. And although some people naturally clear TB -- even those with dangerous strains of *M. tuberculosis* -- Bishai stresses that the situation “is still horrific” in South Africa. “And one of the most sinister sides it displays is that it has infected a number of healthcare workers--the very nurses and doctors who care for the patients,” he says.

The tragedy in Tugela Ferry raised critical scientific and clinical questions. How far has XDR-TB spread? Had HIV infection made people more susceptible to developing TB, and if so, how? What are the immune responses to *M. tuberculosis* in HIV-infected people? Did the drug resistance develop because people had a TB infection and failed to properly take their medicine? Or had they acquired an XDR-TB strain by transmission from others? What enables the lucky few to survive XDR-TB? What’s hampering the development of faster diagnostics, better drugs, and an improved vaccine? What public health measures can slow XDR-TB’s spread in an HIV-infected population?

Perhaps most importantly, Tugela Ferry forced researchers to confront their own shortcomings. “Sorting out exactly how those two pathogens are interacting with each other really has not been studied enough,” says HIV/AIDS immunologist Bruce D. Walker, an HHMI investigator at Massachusetts General Hospital and Harvard Medical School in Boston. “These diseases have always been studied separately, even though clinically they’re completely intertwined. The science has been done independently by different groups that have little interaction with each other.” Walker, with help from HHMI and several other funders, is one of the researchers leading the movement to bridge these gaps.

**Birth of K-RITH**

In 2006, Walker spoke with then HHMI President Thomas Cech about developing an international project. HHMI had never focused on global health, but he says Cech was intrigued by a successful HIV research project Walker had helped launch in Durban with UKZN and the Doris Duke Charitable Foundation. “Tugela Ferry clearly had an impact on HHMI's thinking about this project,” says Walker. “They visited there and other sites, and saw the need and opportunity.”

HHMI has committed $60 million over 10 years to the project, which includes a new 7-story, 40,000 square-foot building on the UKZN campus that features several biosafety 3 laboratories, which can safely handle dangerous pathogens like XDR-TB. “It’s beautiful, breathtaking,” Bishai said in August, as the construction crews were putting on the finishing touches. “I try to give as many tours as I possibly can. It’s nicer than my lab at Johns Hopkins.”

Bishai’s research has focused on the molecular genetics of tuberculosis and its interactions with the immune system. He specifically has investigated the regulatory genes carried by *Mycobacterium tuberculosis* that allows it to lie dormant for years and then reactivate. “He invented the field,” says Chaisson of Hopkins, who has collaborated with Bishai.

Bishai has hired six investigators with “world-class careers” to work at K-RITH, and hopes to bring on four more. “Every one comes with ties to big programs in developed countries,” he says. Their specialties include everything from studying how *M. tuberculosis* thrives in human cells to the genetics of the bug and developing new diagnostics with microfluidics. “The enabling of top-notch human biology at the epicenter of TB and HIV epidemics is synergistic,” says Bishai “It could change the mode of other global health programs.”

**An Urgent Need for Better TB Tests**

*Mycobacterium tuberculosis* is transmitted through the air and infects about one-third of the world’s population. It causes no harm to 90 percent of those infected, remaining latent inside the lungs, hiding in masses of immune cells called
granulomas that Bishai studies intensively. But more than nine million people each year develop active cases of TB, which typically cause weight loss, night sweats, and lung damage. Nearly two million people die each year from what should be a curable disease, either because they are not properly diagnosed, do not take the right medications, or have drug-resistant strains that defy treatment.

Roughly half a million people with TB have drug-resistant strains; according to a 2010 global report by the World Health Organization (WHO), 58 countries had reported at least one case of XDR-TB. WHO estimated in 2011 that some 25,000 cases of XDR-TB emerge each year. A study published online by The Lancet on August 30, 2012, analyzed MDR-TB patients in South Africa and eight other countries and found that between 2005 and 2008, 6.7% had XDR-TB.

These numbers may well underestimate the true scope of the problem; according to WHO, only 65% of new TB cases are identified and reported to them, and that says nothing about drug-resistance. Kristina Wallengren, who is advising K-RITH on clinical research projects, published a study in 2011 showing that the incidence rate of MDR-TB in KwaZulu-Natal is at least two times higher than reported. “We have very limited information about the epidemics of XDR-TB and MDR-TB,” says Gerald H. Friedland, an epidemiologist at Yale University who led the study of the Tugela Ferry outbreak. “It’s hard to do battle when you don’t know the size and location of the other army.”

The biggest obstacle faced by epidemiologists and clinicians is that their diagnostic tool kit is rusty, making it difficult both to detect infections and drug resistance. “We’re using things we were using 30 to 40 years ago,” says Karim.

To begin the laborious diagnosis of TB, patients first cough up a sputum sample. Lab technicians then stain the sputum with a dye, wash it with an acid, and examine it under a microscope to check for the presence of TB bacteria. The test has a high false-negative rate, and it’s even more misleading in HIV-infected people, who often have little disease in their lungs but life-threatening M. tuberculosis infections in their abdomens, brain and other hard-to-test regions of the body.

If the sputum does not have detectable TB bacteria under the microscope, it must be grown in culture dishes or special liquid to confirm the diagnosis. Because TB grows slowly, this can take two to six weeks. A third test assesses drug resistance. This test also has serious practical limitations. Again, the first obstacle is time. Technicians must sprinkle the culture dishes with various drugs to determine which ones can kill the TB growth. This takes several weeks as well. The long process means patients may die from XDR-TB before they receive the results—as happened in Tugela Ferry. This test also is difficult to do in South Africa and many other cash-strapped countries, as it requires special high-tech laboratories that minimize the risk of M. tuberculosis escape, as well as elaborate protective gear for the technicians handling the material.

Ultrasensitive tests such as the polymerase chain reaction assay (PCR) that amplify tiny bits of genetic material promise to revolutionize TB diagnostics, and one, GeneXpert, now is on the market that can detect TB and resistance to rifampicin, a staple of first-line treatment. Endorsed by the WHO in December 2010, GeneXpert places a sputum sample in a disposable plastic cartridge that contains the needed reagents, produces a result within 2 hours, and is safer to run than the standard culture assays. “It’s setting the stage for a game change,” says Bishai.

Not only does GeneXpert promise to improve treatment, Bishai says it can greatly help researchers. “GeneXpert changes things enormously because you can quickly ascertain the likely MDR patients from the likely drug-susceptible ones,” says Bishai. “In a number of drug studies, we want to enroll drug-susceptibles
or MDR patients, and now you can figure it out right in the clinic. It saves enormously on the cost of the study.”

Yet one of the barriers to use of GeneXperts has been cost. The manufacturer--Cepheid of Sunnyvale, California--this August agreed to “concessional” pricing that cut prices on cartridges by 40% (to $9.98 each) for 145 countries with high TB burdens or developing economies. The Foundation for Innovative New Diagnostics, a nonprofit based in Geneva, also negotiated concessional pricing of $17,000 for machines that can run up to 20 tests in an eight-hour shift. UNITAID, another Geneva-based nonprofit, helps fund the purchase of GeneXpert machines and cartridges, and says scaling it up could detect as many as 700,000 new cases of TB each year.

GeneXpert has limitations aside from costs, Bishai notes. Ideally, patients -- even in rural clinics -- should receive “point-of-care” tests that take, say, 20 minutes and give results before they leave. William R. Jacobs, Jr., an HHMI investigator at Albert Einstein College of Medicine in, New York, says another key drawback of such tests is that they can only find what they’re looking for—and the known resistance mutations that PCR can detect do not explain all of the drug failure seen in the clinic.

Identifying drug resistance mutations in TB is slow, tedious work. Isoniazid, the most commonly prescribed first-line drug for TB, and the best understood of the dozen or so drugs used to treat TB, has been used since 1952. It wasn’t until the early 1990s that researchers -- including one team led by Jacobs -- reported how isoniazid actually works and subsequently began finding mutations that make the bacteria resistant to the drug. And Jacobs notes that no mutations have yet been found for about 15 percent of M. tuberculosis strains that can dodge isoniazid.

It requires a tremendous amount of work to prove that a mutation causes resistance. “Even if you find a mutation, you’ve got to transfer it from the drug-resistant strain to a drug-sensitive strain,” says Jacobs. “That’s the only way to prove that resistance is bona fide.” That process again requires growing cultures of the mycobacteria, which can take a few months. And strains that develop drug resistance are, by definition, less fit—otherwise the mutant would have occurred naturally—and are notoriously slow growers.

For several years, Jacobs’ lab has worked on developing inexpensive tests that sidestep many of these problems. In an interesting strategy that turns the biological tables on the tubercle bacillus, Jacobs’ lab has been trying to develop highly sensitive diagnostic tools with the help of viruses that grow in healthy bacterial hosts. Jacobs engineered reporter genes into the virus genome that, when expressed, make the host glow if the viruses grow; glowing bacteria in the presence of a drug provide a quick and highly sensitive assay for drug-resistant bacteria. He also hopes his phage system will make it easier for researchers to detect tiny amounts of M. tuberculosis in a sputum sample, identifying many infected people now missed by the current diagnostic tests.

Bishai is leading a massive sequencing project of XDR-TB strains, which he hopes will uncover mutations that then can be directly plugged into a platform like the GeneXpert test, leading to a day when each clinicians know the drug susceptibility profiles of the bug in each of their patients.

**Prospects for Better Treatments**

In an ideal situation, doctors would quickly identify both HIV and M. tuberculosis infections, assess whether a person harbors drug-resistant pathogens, isolate patients who have MDR- or XDR-TB to reduce the risk that they would infect others, and then prescribe the appropriate drugs. The clinical reality is vastly more complicated and confusing.

Many people who have relatively intact immune systems and no knowledge that they are infected
with HIV seek medical care because they’re having night sweats and losing weight. Clinicians frequently do not suspect that those people are infected with HIV, let alone TB. And even if they do tests to confirm that their patient is HIV-positive, physicians may still miss underlying TB infection. So those patients may receive antiretroviral drugs for HIV, but no TB medication. “If you don’t treat TB in people with HIV, they die,” says Chaisson.

For patients who test positive for both TB and HIV, many clinicians choose to first bring the TB under control. In 2008, Karim headed a study of more than 600 people that showed that patients waiting to complete TB treatment before getting HIV drugs were 50 percent more likely to die than those being treated for both diseases simultaneously.

Multidrug-resistant TB in people with HIV infection adds its own challenges. HIV speeds the course of disease in tuberculosis. It can reactivate a latent infection with *M. tuberculosis* or make it easier for a person to fall sick from a new TB strain. Treatment of regular TB requires six months of one pill that combines several drugs. Treatment for MDR-TB requires two years of treatment with different toxic drugs, some of which must be injected. People with XDR-TB have fewer options still, as they typically do not respond to several second-line drugs. The options are reduced even further when patients are infected with both XDR-TB and HIV.

Tugela Ferry laid bare the challenges faced by clinicians combating XDR-TB. *M. tuberculosis* evolves drug-resistance mutations in part because infected people do not properly take their medications. But the surge of XDR-TB at Tugela Ferry appears to have a different engine behind it. Analysis of the XDR-TB infections among patients in an HIV treatment program at the Church of Scotland Hospital, revealed a dominant strain, indicating that “primary” or transmitted infection -- rather than spotty TB treatment -- was driving the outbreak. What’s more, a careful analysis of 17 patients who were being treated for TB infections and later developed MDR- or XDR-TB infection found that all had been infected by a new strain of TB.

“This has substantial implications,” says Karim. TB recommendations have emphasized strategies, such as directly observed therapy, to reduce the chances of missed doses and the development of resistance. There’s also been a growing call to give HIV-infected people isoniazid as a prophylactic to prevent reactivation of latent TB. But if new infections are the main driver of XDR-TB’s spread, the emphasis switches to old-fashioned, public health tactics such as patient isolation, face masks, and ample ventilation.

Since XDR-TB surfaced in Tugela Ferry, clinicians have become more aggressive about identifying patients with XDR-TB, treating them appropriately, and trying to contain its spread. The mortality rate has dropped to 82 percent. “That looks good only in relation to 98 percent, the original report,” cautions Friedland. “The most important thing at this time is to prevent new infections. Once you’ve got it, the outcome is not going to be great.”

Several new TB drugs are in clinical trials, and two companies—Otsuka Pharmaceutical Company and Janssen Therapeutics—have tested novel compounds in large clinical trials and are seeking regulatory approval (one company in Europe, the other in the United States). These drugs could come to market in 2013. The Global Alliance for TB Drug Development is one of several groups testing combination of drugs that might shorten the course for a cure, which now takes so long that many people fail treatment and develop resistance.

Yet even if these treatments pan out, curing a drug-resistant infection likely will still take many months or even years, which gives *M. tuberculosis* ample time to devise ways around the new weapons. “You can’t win easily against TB,” says Karim. “You solve the problem and it creates another one.”
Vaccines

As with many other infectious diseases, a vaccine is the Holy Grail in TB research, as it might one day send the scourge into obscurity, as with smallpox and polio. But this likely won’t happen anytime soon, according to HHMI’s Walker. For one thing, progress to define the immune responses that protect a person from TB has been slow. “Very little immunology has been done,” he says.

The TB vaccine, Bacillus Calmette-Guérin (BCG), has been in use for 80 years and has been given to about three billion people. The vaccine contains a weakened version of Mycobacterium bovis, which causes TB in cattle. But BCG has a checkered history, working in some studies and completely failing in others.

“Nobody knows what protects us from TB,” says Chaisson. “And that’s important: If you’re developing a TB vaccine, you want to know what you’re trying to promote.”

Several TB vaccines are in early human studies, but all aim to fortify the old-fashioned vaccine rather than to trigger novel immune responses. To that end, Walker has tried to shed light on one of the most vexing mysteries: How does the immune system contain a TB infection? He looked at HIV-infected people in Durban who also had occult M. tuberculosis infections. He hoped that examining the interaction of both bugs would reveal a clearer picture of the relevant immune responses. “The immune system must be doing something to contain TB,” says Walker. “When it fails, you get reactivation.”

CD4 T cells form the basis of immunologic “memory” to orchestrate an immune response if they have earlier confronted an invader like TB. Walker’s study indicates that CD4s work overtime to contain the TB infection. HIV, in turn, specifically targets and destroys CD4 cells, and he found that people with higher levels of the virus had more impaired TB-specific CD4 responses. A new study will ask whether treating an HIV infection can restore these TB-specific responses and prevent that disease.

HIV now infects an estimated 34 million people, one-third of whom already are infected with M. tuberculosis. Most of these infections remain contained, but for how long? And as Tugela Ferry dramatically demonstrates, HIV leaves people much more susceptible to new infections with more dangerous strains of M. tuberculosis than humans have ever faced.

Bishai believes K-RITH, located in one of the world’s most accommodating environments for M. tuberculosis, can play a leading role in helping to address the deadly duo of HIV and TB. “We’ll have ready access to clinical specimens that enable us to focus on true biomarkers for diagnosis that would be difficult to obtain in Baltimore, Maryland,” says Bishai. “We’ll also be able to coordinate trials for new therapeutics and vaccines.” And he expects to share the M. tuberculosis specimens they collect with researchers everywhere. “I’m very optimistic that we can get people interested who wouldn’t otherwise think about tuberculosis and HIV,” he says. That, by itself, could go a long way to solving the seeming intractable problems that dog the field.

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