Antibiotic Treatment of Tuberculosis: Old Problems, New Solutions

With tuberculosis and drug resistance surging, the search is on for new drugs along with better and faster ways of evaluating them

Sanjay K. Jain, Gyanu Lamichhane, Sridhar Nimmagadda, Martin G. Pomper, and William R. Bishai

The German physician and scientist Robert Koch presented his discovery of *Mycobacterium tuberculosis* on March 24, 1882, noting:

"If the importance of a disease for mankind is measured by the number of fatalities it causes, then tuberculosis must be considered much more important than those most feared infectious diseases, plague, cholera and the like. One in seven of all human beings dies from tuberculosis. If one only considers the productive middle-age groups, tuberculosis carries away one-third, and often more."

More than 125 years later, tuberculosis (TB) is surging, leading to more deaths in 2006 than any previous year in the last few decades. Damage from this disease continues to grow despite effective therapies for drug-susceptible TB that keep the incidence of TB in Western countries at record lows. The reasons for this shocking failure to control TB globally pivot on the difficulty of providing sustained, properly dosed, multiantibiotic therapy in developing countries. Worse, this failure has led to development of drug-resistant TB, including the recent recognition of extensively drug-resistant (XDR) TB in precisely those regions that are least equipped to deal with it. The result, though predictable, is no less disturbing: high rates of rapidly fatal TB with an estimated 1.7 million deaths worldwide annually, 9.2 million new cases of TB disease, and more than 2 billion people infected with "latent" TB.

"Short-Course" Therapy To Treat TB: a Paradox

Standard anti-TB therapy typically continues for six months. For the first 2 months, patients receive three to four drugs, namely rifampin (R), isoniazid (H), pyrazinamide (Z), and, in some cases, ethambutol (E). During the final 4 months, they continue with rifampin and isoniazid (Fig. 1).

This six-month regimen paradoxically

Summary

- Tuberculosis, particularly extensively drug resistant, or XDR-TB, is surging, and experts estimate 1.7 million deaths worldwide per year and 9.2 million new cases per year from TB, along with more than 2 billion people having latent infections.
- Standard anti-TB therapy typically continues for six months and requires patients to take about one-third of a kilogram of a mixture of anti-TB drugs when administered daily.
- While testing candidate TB drug regimens in mice is economical, reproducible, and popular, human clinical trials have not always matched the results from mice—a large moxifloxacin trial being a case in point.
- Several biomarker technologies, including monitoring of mRNA and inflammatory response molecules as well as imaging with positron emission tomography and single-photon emission computed tomography, are under development to boost the speed and improve the reliability of testing anti-TB drug candidates.
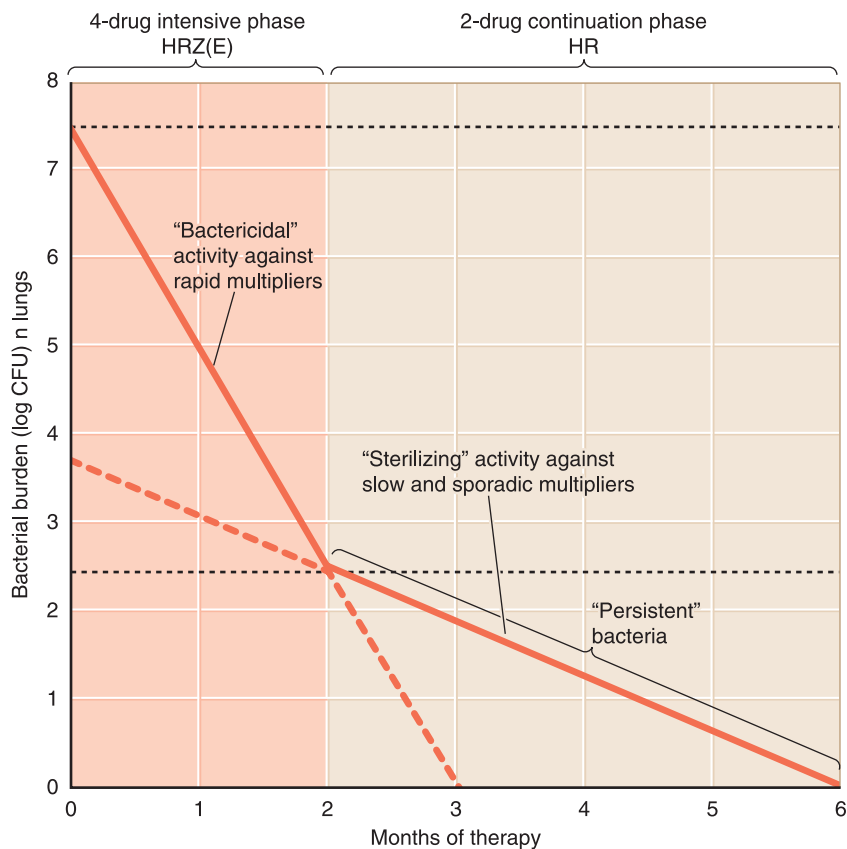
Sanjay K. Jain is Assistant Professor in the Department of Pediatrics, Gyanu Lamichhane is Assistant Professor in the Department of Medicine, Sridhar Nimmagadda is Postdoctoral fellow in the Department of Radiology, Martin G. Pomper is Professor in the Department of Radiology, and William R. Bishai is Professor in the Department of Medicine at the Johns Hopkins University School of Medicine, Baltimore, MD.

FIGURE 1

4-drug intensive phase
HRZ(E)

2-drug continuation phase
HR

"Bactericidal" activity against rapid multipliers

"Sterilizing" activity against slow and sporadic multipliers

"Persistent" bacteria

Bacterial burden (log CFU) n lungs

Months of therapy

Standard anti-TB therapy. Current model of short-course therapy for tuberculosis showing the intensive (bactericidal) phase in the first 2 months during which rapid killing is observed and the inclusion of pyrazinamide is necessary for long-term treatment shortening. Typically, optimal regimens in this phase require four drugs: isoniazid (H), rifampin (R), pyrazinamide (Z), and ethambutol (E). The continuation (sterilizing) phase during the remaining 4 months is shown where the rate of killing is lower due to persister bacteria which are slowly or sporadically multiplying. If the isolate is susceptible, Z and E are dropped in the continuation phase. In abbreviated form, standard therapy in humans is $HRZE_2$-HR_4. (Adapted from J. Grosset, Clin. Chest Med. **1:**231–241, 1980).

cally replicate. Cells falling into these separate populations are killed biphasically, with the rapid multipliers being cleared during the earlier bactericidal phase, and the slowly and sporadically multiplying organisms killed during the later sterilizing phase (Fig. 1).

Isoniazid and streptomycin (S) have good early bactericidal activity when tested in animals, reducing infection burdens initially by four to five orders of magnitude. However, these agents do not clear the infections. Thus, despite the early rapid kill, the slowly and sporadically multiplying populations of *M. tuberculosis* remain as "persisters," and these may only be cleared by antibiotics possessing "sterilizing" activity.

Such persister cells are more slowly cleared and may be tolerant (phenotypically resistant) to anti-TB therapy. Treatment with rifampin, a sterilizing antibiotic, gradually reduces bacillary counts to zero during the latter phase of therapy in humans. When pyrazinamide is administered during the first two months of treatment, it shortens the overall length of therapy, albeit without showing much bactericidal activity when used on its own. Ethambutol, superfluous in treating drug-susceptible *M. tuberculosis*, is a drug that is routinely included in the regimen when particular populations are believed to harbor drug-resistant TB.

also is called "short-course" anti-TB therapy. When administered daily, a typical adult patient is required to consume more than one-third of a kilogram of anti-TB drugs in a series of 182 daily doses—in contrast to 2–5 grams in 5–7 doses for curing an uncomplicated, community-acquired bacterial pneumonia or similar infection.

So why does it take so long to treat TB disease in humans when cultures of *M. tuberculosis* may be killed in a matter of mere hours to days? According to current models, several populations of bacilli have to be dealt with in infected individuals: those that are growing rapidly, those growing slowly, and those sporadi-

Candidate Drugs For Treating TB

While prospects for new broad-spectrum antibacterials are limited, several promising narrow-spectrum anti-TB drugs are under development (Table 1). Among those new agents are two nitroimidazoles, PA-824 (Pa) being developed by the TB Alliance and Chiron, OPC-67683 (O) by Otsuka, a diarylquinoline TMC-207 by Tibotec, an ethambutol derivative SQ-109 by Sequella, and a pyrrole LL-3858 by

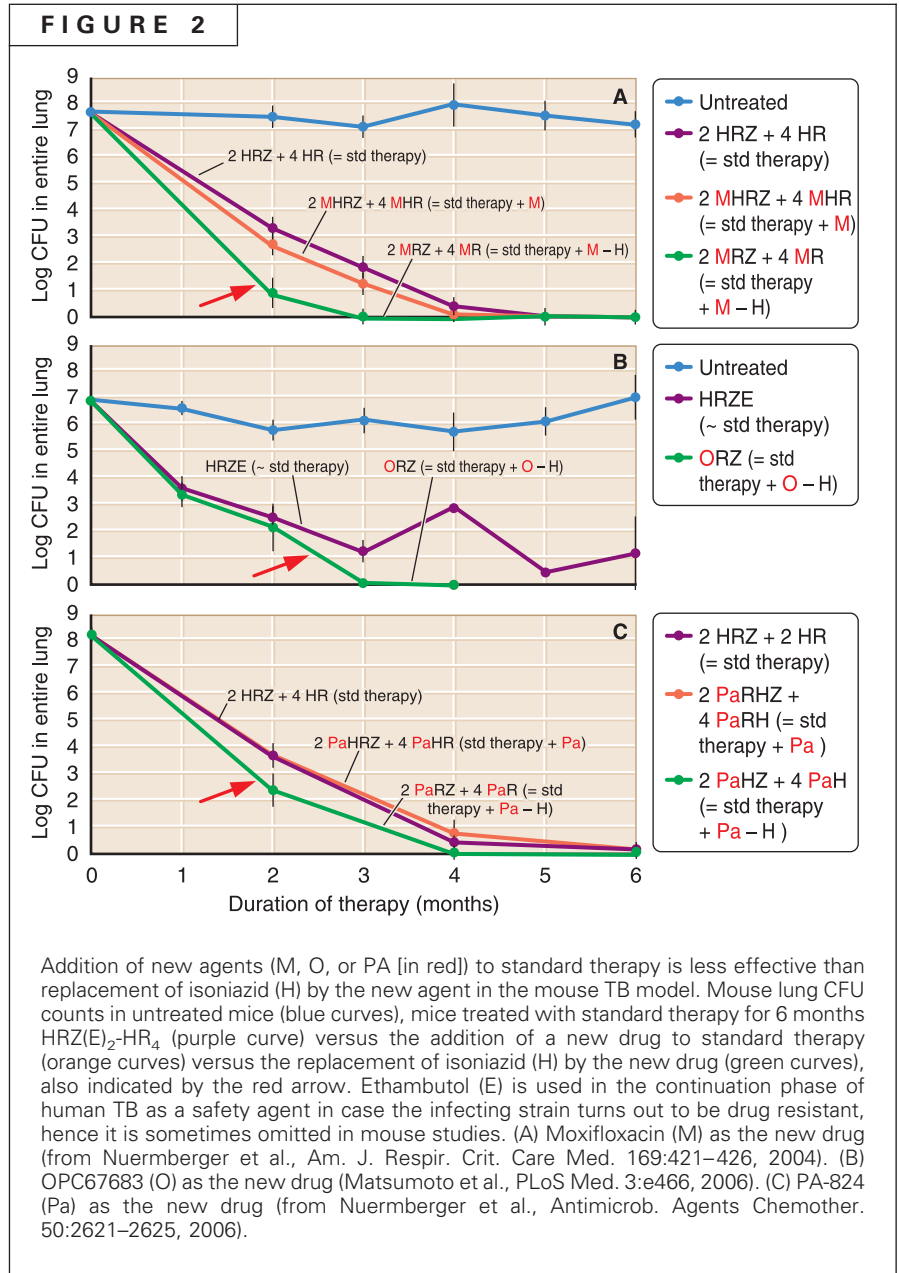Lupin. Several of them are entering phase II/III clinical trials.

Concerned over our vulnerability to multidrug-resistant (MDR) and XDR TB, experts also are reexamining approved antibacterials that might be used to treat patients with TB. This list includes the fluoroquinolone antibiotics moxifloxacin (M) and gatifloxacin (G), the oxazolidinone antibiotic linezolid, the nitroimidazole antibiotic metronidazole, and the long-acting rifamycin rifapentine (P), which is licensed for TB. One near-term research priority is to determine whether agents such as fluoroquinolones and rifapentine may be used in combinations that offer advantages over standard anti-TB therapy.

Considerable preclinical testing is required to identify the multidrug regimens that best exploit the activities of new agents. While in vitro studies help, assessing bactericidal and sterilizing activities in infected animals that receive doses equivalent to what humans could expect is an essential part of these evaluations.

Insights from Evaluating Novel TB Drug Combinations

Evaluating new agents combined with established anti-TB drugs in mice often brings surprises. For example, adding one of several newer agents, including moxifloxacin, PA-824, and OPC-67683, to standard anti-TB drug regimens only marginally reduced TB organ burdens. Thus, the new agent plus the standard regime of HRZ is little better than HRZ without the new agent.

One drug in the standard mix, isoniazid, complicates these tests in mice. Remarkably, omitting it from such studies sometimes allows other antibacterial drugs—for example, moxifloxacin—to stand out in terms of reducing *M. tuberculosis* organ burdens and the time it takes to cure the animals being tested (Fig. 2). Thus, although isoniazid is a potent bactericidal agent, it appears to antagonize the actions of other anti-TB drugs such as rifampin and pyrazinamide that are being administered simulta-

neously (consistent with Fig 2A-C). Replacing isoniazid—and thus avoiding antagonism that it causes—with similarly acting drugs such as moxifloxacin, PA-824, or OPC-67683 reveals the possibility of shortening treatments. In particular, substituting moxifloxacin for isoniazid produces a sustained cure in mice after only 4 months of treatment, with no relapses 90 days after anti-TB therapy ends. Clinical trials are under way to address whether these results in mice hold true for humans.

FIGURE 2

Addition of new agents (M, O, or PA [in red]) to standard therapy is less effective than replacement of isoniazid (H) by the new agent in the mouse TB model. Mouse lung CFU counts in untreated mice (blue curves), mice treated with standard therapy for 6 months HRZ(E)$_2$-HR$_4$ (purple curve) versus the addition of a new drug to standard therapy (orange curves) versus the replacement of isoniazid (H) by the new drug (green curves), also indicated by the red arrow. Ethambutol (E) is used in the continuation phase of human TB as a safety agent in case the infecting strain turns out to be drug resistant, hence it is sometimes omitted in mouse studies. (A) Moxifloxacin (M) as the new drug (from Nuermberger et al., Am. J. Respir. Crit. Care Med. 169:421–426, 2004). (B) OPC67683 (O) as the new drug (Matsumoto et al., PLoS Med. 3:e466, 2006). (C) PA-824 (Pa) as the new drug (from Nuermberger et al., Antimicrob. Agents Chemother. 50:2621–2625, 2006).

Table 1. Anti-TB drugs available and in development

Anti-tuberculosis drugs in use	Date first used	Anti-tuberculosis drugs under development	
		Drugs under study as anti-TB agents[a]	New drugs in pre-clinical development
Streptomycin	1945	Moxifloxacin (fluoroquinolone)	PA-824 nitroimidazole (TB Alliance, Chiron)
Para-amino salicylate (PAS)	1946	Gatifloxacin (fluoroquinolone)	OPC-67683 nitroimidazooxazole (Otsuka)
Thiacetazone	1946	Linezolid (oxazolidinone)	TMC-207 diarylquinoline (Tibotec)
Isoniazid	1952	Metronidazole (nitroimidazole)	SQ-109 ethambutol derivative (Sequella)
Pyrazinamide	1952		LL-3858 pyrrole (Lupin)
Cycloserine	1955		
Ethionamide	1958		
Capreomycin	1960		
Ethambutol	1963		
Rifampin	1967		
Rifapentine[b]	1999		

[a]Approved for human use in other infections.
[b]Intermittent use once or twice per week only.

Meanwhile, recent studies suggest that current rifamycin dosing for patients with TB may be too low. However, the development of rifamycin derivatives with long half-lives, including rifapentine, rifabutin, and rifalazil, makes it possible to raise the effective dosing. Of this group, rifapentine has a 10–15 hour half-life, which is about fivefold longer than that of rifampin, and it has a two- to fourfold increase in in vitro activity against *M. tuberculosis*. There is now considerable interest in using it daily or increasing the twice-weekly dose as a means of boosting rifamycin exposure. For example, substituting daily rifapentine for rifampin signifi-

Table 2. Common clinical trial modalities and endpoints for TB treatment trials

Early bactericidal activity (EBA)[a]	Sputum culture and smear conversion rates[b]	Phase III proof-of-cure studies[c]
Can test monotherapy with new drug	May be adequate proof-of-efficacy with some regulatory authorities	Considered the gold-standard for efficacy with regulatory agencies
Good for drugs with high bactericidal potential, may underestimate for drugs with high sterilizing potential	New drug must be tested as part of a multidrug regimen deemed to be ethical	New drug must be tested as part of a multidrug regimen deemed to be ethical
High degree of patient-to-patient variability	Good for drugs with high bactericidal potential, but may underestimate for drugs with high sterilizing potential	Costly (for adequate power requires approximately 500 patients or more)
Requires inpatient facility		Time-consuming: minimum of 3 years
Requires experienced lab	Problems with lab-to-lab variability in limit of detection for smear and culture	
Expectorated sputum CFU counts show weak correlation with radiologic disease	Expectorated sputum CFU counts show weak correlation with radiologic disease	
Unknown correlation with cure		

[a]Newly diagnosed pulmonary TB patients receive a short (1–2 week) course of experimental drug and quantitative sputum is collected each day and evaluated for CFU counts.
[b]During the initial phase of therapy expectorated sputum is sampled at 2 week intervals until week 8 and monitored for conversion to AFB smear negativity and mycobacterial culture negativity
[c]Population treated with full courses of test and standard regimens and monitored for relapse for at least two years.

cantly shortens anti-TB therapy needed for relapse-free cures of mice.

Studying Drugs in Mice with TB: Useful, Not Perfect

While testing new TB drug therapies in mice is economical, reproducible, and popular, the key question is how well it predicts performance in humans. Over several decades, this approach often proved successful in predicting human efficacy. For example, pyrazinamide became an essential component of modern multidrug therapy after being screened in mice. More recently, the sterilizing activity of RZ combination regimens in mice led to tests in humans showing RZ efficacious as 2-month, short-course preventive therapy for latent TB. However, this regimen is no longer used because it induces idiosyncratic hepatitis in humans.

Some TB clinical trials are focused on drug combinations to shorten the treatment period. For example, Study 27 in the Centers for Disease Control and Prevention (CDC)-sponsored TB Trials Consortium (TBTC) evaluated moxifloxacin as a substitute for ethambutol (E) during the initial phase of therapy. Patients were randomized to receive HRZE or HRZM during the first 8 weeks of therapy. Those patients who received moxifloxacin progressed more quickly to negative sputum cultures, although the overall percentage of patients who achieved culture-negativity by 8 weeks was nearly the same. The clinical trial results closely resembled those in mice, where HRZM was marginally better than HRZ (Fig. 2A). However, in a more recent clinical study in Brazil, HRZM not only accelerated but also increased the proportion of patients with negative sputum cultures at 8 weeks compared to HRZE.

Another regimen that looked more exciting when tested in mice, replacing isoniazid with moxifloxacin, was also tested in humans as TBTC Study 28: initial phase MRZE versus HRZE. However, the human results did not resemble those in mice in this trial, and there was no statistically significant difference between the two at 8 weeks. Why?


FIGURE 3
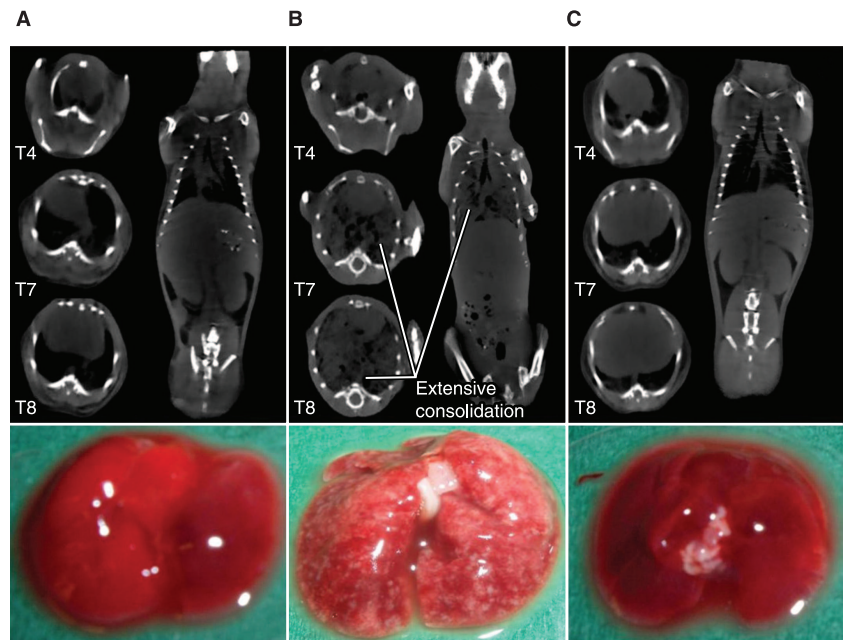
High-resolution CT scans to evaluate tuberculosis disease in the mouse model. (A) Uninfected mouse. (B and C) Aerosol infected (*M. tuberculosis*) mice with 8.3×10^3 CFU implanted in lung at day 1. (B) Four weeks after aerosol infection, the untreated mouse demonstrates extensive lung consolidation with lung CFU of 2.1×10^8: (C) Four days after aerosol infection, mouse from the same experiment was administered daily INH (25 mg/kg) for 3 weeks. On the day of sacrifice, the lungs demonstrate minimal to no consolidation with lung CFU of 6.5×10^1. Lower panels show the corresponding gross lung pictures.

One possibility is that that the divergence between results of MRZ(E) versus HRZ(E) stems from the fact that human sputum culture conversion rates may not be directly comparable to the parameters measured in mice—namely, total bacterial organ burdens over time. Second, isoniazid antagonism of other drugs could be of much smaller magnitude in humans than mice. Third, TB-inflicted tissue damage may be less severe in mice than in humans, monkeys, or rabbits, thereby giving faster CFU clearances in some regimens. Fourth, mice are more susceptible to TB than humans, developing chronic multibacillary infection even after small inocula, whereas humans tend to contain the initial inocula and develop latent infections. Fifth, mice clear some antibiotics very quickly compared to humans and these human pharmacokinetics/pharmacodynamics parameters of antibiotics are difficult to emulate in some instances. Hence, despite increased costs and biohazard

FIGURE 4
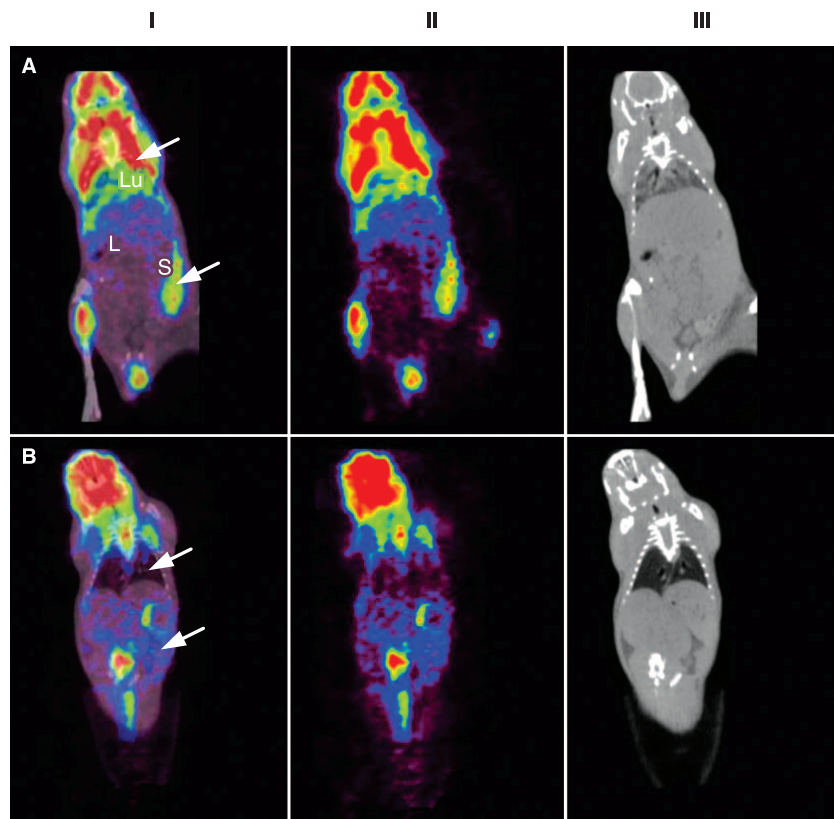
PET scans to evaluate tuberculosis disease in the mouse model. [^{18}F]FDG-PET showing inflammation in lungs of an *M. tuberculosis*-infected mouse. Coronal sections of mice by the following modalities: Column I: fused CT+PET. Column II. PET alone. Column III. CT alone. Row A. Mouse infected intravenously with *M. tuberculosis* H37Rv evaluated 4 weeks after infection. Row B. Uninfected mouse. Unlike the uninfected mouse, [^{18}F]FDG was taken up by the lung (Lu), spleen (S) and liver (L) in the infected mouse depicted by the fused CT+PET image in row A. Mean lung activity was 94.5 Cts/s/pix for infected mouse, compared with 25.0 for uninfected mouse. The white arrows point out the lungs and spleens.

inconveniences, there may be value in testing certain aspects of anti-TB drug performance in larger animals such as guinea pigs, rabbits, and nonhuman primates.

Surrogate Markers Would Help in Evaluating Anti-TB Therapies

Tools for evaluating TB treatments, especially in clinical trials, are painfully primitive, expensive, and time-consuming. For instance, multidrug cocktail phase III trials entail treating hundreds of patients with full courses of standard and test regimens and monitoring them for at least two years for relapses (Table 2). Another option, phase IIB studies monitoring patients for 8 weeks for sputum culture conversion rates, is of limited value because sputum bacterial burden does not always correlate closely with overall disease.

Contrast TB clinical trials options with those for testing antiviral drugs against HIV, where there are well-validated markers of disease burden, namely quantitative viral loads, and progression of disease that is measured in declining CD4 cell counts.

In recognizing this need, officials at CDC and other experts call for urgent development of new markers and endpoints for TB. However, such biomarkers will be useful only if they refine and simplify efforts to evaluate candidate drugs or reduce time for measuring responses to them. Further, biomarker technology needs to be validated before surrogate markers can be used to measure endpoints. Biomarker technologies that are validated in animals and easily scalable to humans may lay the groundwork for evaluating candidate anti-TB therapies in patients.

Several biomarker technologies are under development for TB. For instance, specific mRNA molecules are being used to monitor mycobacterial viability. In one series of studies, investigators used antigen 85B mRNA, which was lost rapidly when anti-TB therapy was started. Thus, changes in levels of this mRNA species correlated with culture clearance of the pathogen. Moreover, for one patient who subsequently relapsed, that mRNA remained persistently detectable. Other approaches measure nonspecific markers of inflammation such as erythrocyte sedimentation rate, C-reactive protein, β2-microglobin, and pro-calcitonin, to follow TB and other bacterial diseases in humans. In principle, following any of these markers would be useful for assessing responses to anti-TB therapy because host immunity is essential for controlling TB and for suppressing reactivation.

In terms of immunological biomarkers, the T cell-based interferon γ (IFN-γ) release assays (IGRAs) are perhaps the most developed. These assays measure IFN-γ-release by T cells in response to two *M. tuberculosis* antigens, early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10), both of which are strong targets of T helper type 1 cells in TB patients. Commercial kits, such as the QuantiFERON-TB Gold In Tube by Cellestis that measures IFN-γ levels in whole blood and the T-SPOT.TB by Oxford Immunotec that is an ex vivo enzyme-linked immunospot assay for counting antigen-specific T-cells, are available.

These assays provide reliable rapid results even when performed on extrapulmonary specimens. However, IGRA findings can vary among different populations and regions. Also, how long T-cell responses remain elevated in patients who are treated for latent disease is not known. Positive results from IGRAs, which were developed for diagnosing latent infections, show delays when patients convert to active disease. They also cannot measure cases of cured disease. Also, even though available in kits, these assays remain resource intensive, particularly for use in resource-poor countries. Nonetheless, these assays may provide useful biomarkers for evaluating novel anti-TB therapies.

Imaging Biomarkers

Traditional radiographic tools, including chest radiography and computed tomography (CT), lack sensitivity and specificity for monitoring responses to anti-TB therapies. However, positron emission tomography (PET) with radiolabeled [^{18}F]-2-fluoro-deoxy-D-glucose (FDG), which is being used for diagnosing several types of cancer, may also be used to produce images of tuberculous lesions in humans.

Our preliminary data suggest that serial computed tomography (CT) and FDG-PET are useful for evaluating anti-TB therapy in animals (Fig. 3 and 4). Coregistering high-resolution (50

FIGURE 5

Uptake of radiotracer [^{125}I]FIAU by recombinant mycobacteria and use of the same tracer in vivo for SPECT imaging in mice. (Left) [^{125}I]FIAU uptake by *M. smegmatis* strain expressing thymidine kinase (TK) under the control of the *hsp60* promoter. Both the recombinant *M. smegmatis* (TK) and the wild-type (WT) strains were incubated with 1 μCi of [^{125}I]FIAU for 2 hours. The *M. smegmatis* TK strain actively accumulated [^{125}I]-FIAU compared to the WT strain with mean activity of 34509 (± 2315) and 636 (± 228) counts per minute respectively. (Right) Planar [^{125}I]FIAU-SPECT image. Unlike activity from the WT strain, activity from the TK strain is clearly visualized in the thigh (arrows). Mean activity was 264.0 counts/pixel for the TK infected thigh, compared with 136.5 (~background) for the WT infected thigh. As expected, the thyroid (Th), liver (L), gall bladder (GB) and urinary bladder (UB) take up activity. The UB was blocked by placing a narrow lead strip under the mouse.

μm) CT and PET scans may also help in evaluating host tissue inflammatory indices in granulomas. However, neither of these technologies is yet validated for making preclinical or clinical assessments. Further, because they measure inflammation in host tissues, these lagging indicators may prove slow for detecting bacteriological responses to novel therapies.

In an ongoing effort to detect microbial abundance radiographically, we and others recently selectively imaged bacteria in mice using 1-(2′deoxy-2′-fluoro-β-D-arabino furanosyl)-5-[^{125}I]iodouracil ([^{125}I]FIAU), a nucleoside substrate for bacterial thymidine kinase (TK). Microbial TK enzymes phosphorylate FIAU, which selectively accumulates in bacterial DNA because this labeled precursor is a poor substrate for mammalian TK. Thus, bacteria
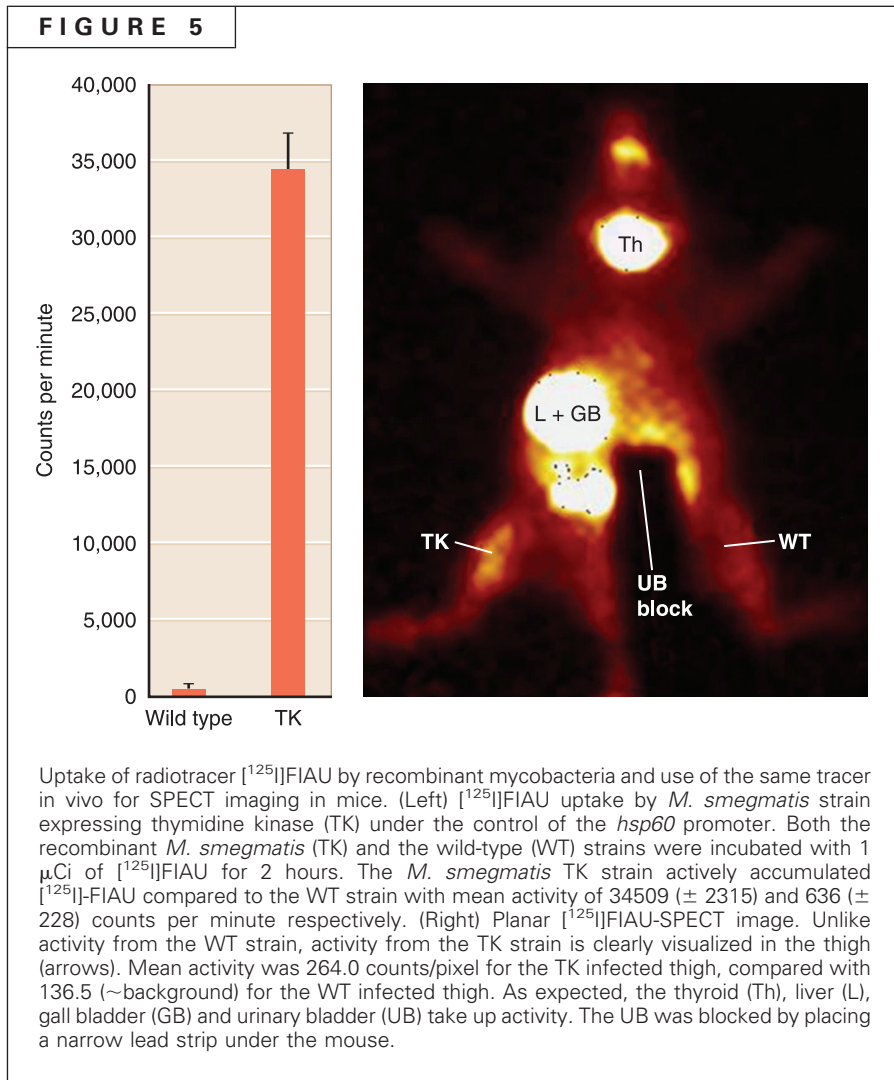
may be imaged and their burden in infected hosts quantified using [^{125}I]FIAU-single photon emission computed tomography (SPECT). We applied this technology to image a *M. smegmatis* strain that was engineered to express TK. This strain actively accumulates [^{125}I]FIAU and may be imaged in mice (Fig. 5). We are continuing to evaluate *M. tuberculosis* strains that are engineered to express TK. We anticipate that [^{125}I]FIAU-SPECT will be helpful for measuring mycobacterial loads in *M. tuberculosis*-infected mice or other animals. Coregistering such images with CT may enable us to determine in which organs and tissue these bacteria accumulate and whether they localize to granulomas or other types of specific lesions. The development of radiological biomarkers is exciting, and this technology seems particularly well suited for tracking infectious pulmonary diseases such as TB, where sampling microbial populations is notoriously difficult.

SUGGESTED READING

Bettegowda, C., C. A. Foss, I. Cheong, et al. 2005. Imaging bacterial infections with radiolabeled 1-(2'-deoxy-2'-fluoro-beta-D-arabinofuranosyl)-5-iodouracil. Proc. Natl. Acad. Sci. USA **102:**1145–1150.

Dorman, S., J. Johnson, N. Padayatchi, et al. 2007. Moxifloxacin vs. isoniazid in the first 2 months of treatment for pulmonary tuberculosis. In: ICAAC. Chicago, 2007

Efron, A., et al. A randomized, placebo-controlled trial of moxifloxacin vs. ethambutol in the initial phase of tuberculosis therapy in Brazil. In: ICAAC. Chicago, 2007

Flynn, J. L. 2006. Lessons from experimental *Mycobacterium tuberculosis* infections. Microbes Infect. **8:**1179–1188.

Gandhi, N. R., A. Moll, A. W. Sturm, et al. 2006. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet **368:**1575–1580.

Nuermberger, E. L., T. Yoshimatsu, S. Tyagi, R. J. O'Brien, A. N. Vernon, R. E. Chaisson, W. R. Bishai, and J. H. Grosset. 2004. Moxifloxacin-containing regimen greatly reduces time to culture conversion in murine tuberculosis. Am. Rev. Respir. Crit. Care Med. **69:**421–426.

Perrin, F. M., M. C. Lipman, T. D. McHugh, and S. H. Gillespie. 2007. Biomarkers of treatment response in clinical trials of novel anti-TB agents. Lancet Infect. Dis. **7:**481–490.

Rosenthal, I., M. Zhang, K. Williams, C. Peloquin, W. Bishai, R. Chaisson, J. Grosset, and E. Nuermberger. 2007. Daily dosing of rifapentine cures tuberculosis in three months or less in the murine model. PLoS Med. **4:**e344.

Spigelman, M., and S. Gillespie. 2006. Tuberculosis drug development pipeline: progress and hope. Lancet **367:**945–947.

World Health Organization. WHO report 2008: Global tuberculosis control—surveillance, planning, financing. Available at http://www.who.int/tb/publications/global_report/2008/summary/en/index.html. Last accessed April 15, 2008.