Grand Rounds:
Fluoroquinolones for TB: Wounded but Not Dead
November 4, 2015

What I'd like to do with our time is tackle the question of where we are with fluoroquinolones coming on the heels of some Phase Three studies that got published last year. And I think you'll agree with me in slide two that we're not there yet. It still takes six months to treat TB even in the simple cases of drug susceptible infection. And we need shorter treatment regimens. We'd like to increase the intermittency, that is reduce the burden for directly observed therapy, and we'd like to stop relying as much on older drugs. And I think you're all familiar with the fact that we're losing the use of INH even here in the United States where the case rate is relatively low. About ten percent of infections last year were INH resistant.

So that's the overarching goal, and quinolones may help us in this regard. And I'll come back to this assessment at the very end of the talk to see how we're doing on trying to achieve these three goals.

So I've broken my comments down into four categories, as you see in this slide, and I'd like to spend a bit of time at the beginning talking about the run up to these Phase Three studies and then bring you up to speed on the Phase Three studies in Part Two. Talk a little bit about new quinolone containing experimental regimens, and then raise some issues about special situations where quinolones may or may not be helpful.

So getting to the run up to these Phase Three studies. Quinolones, as David said, have really come into their own. They're widely used in many aspects of infectious diseases. In the marketplace there are first, second, third and fourth generation quinolones, and I'll call your attention to the fact that gatifloxacin and moxifloxacin are in the fourth generation classes. Both of those were introduced into the market in 1999 and 2000. So they've been with us for a while.

And this next slide shows that there have been a few casualties along the way. Sparfloxacin was on the market for four years but was discontinued due to QT prolongation and photo toxicity. And gatifloxacin was on the market for seven years but was withdrawn from North America due to concerns of dysglycemia, that is both high and low blood sugars in individuals receiving gatifloxacin.

Quinolones work by inhibiting the ability of the organism to replicate its DNA. And I won't belabor the mechanisms other than to say they kill from within unlike drugs like isoniazid which damage the cell wall and lead bacteria to burst and release their inflammatory contents, quinolones shut down the ability of the organism to replicate and then make it a sitting duck for the immune system.

The enzymes that are inhibited are DNA Gyrase and topoisomerase, and *M*. tuberculosis has two genes, gyrA and gyrB that encode its DNA Gyrase. And that is a major mechanism of quinolone-resistant TB. Mutations altering the sequence of those enzymes can allow the enzymes to work but not be inhibited by fluoroquinolones. And then a minor drug resistance mechanism is efflux pumps, and both have been identified in tuberculosis quinolone-resistant strains.

Importantly for the purposes of diagnostics, quinolone resistance is a little bit like rifampin resistance. All of the action is in a very small pair of sequences of DNA, the Quinolone Resistance Determining Region, QRDR-A, is only 30 amino acids, about 100 base pairs, easily amplified by PCR. And the same is true for QRDR-B. So we can look forward to good diagnostic accuracy using the wonders of PCR. And as some of you may know, the Hayne test, which is a PCR-based test that can be done directly on sputum, is widely used overseas, can, indeed, detect the majority of quinolone-resistant TB by amplifying those QRDR regions that I just mentioned and then using DNA hybridization slot blot technology to identify whether a strain is resistant or susceptible.

So I'd like to discuss a case before I go on any further to discuss the quinolones and their role in TB and how we got up to 2014.

So here's a 62-year-old man with dyspnea, weight loss and fever. He's had it for two months. And that's the actual gentleman there sitting next to me. He had lost 60 pounds. Was unable to complete sentences.

He was so dyspneic he couldn't climb a flight of stairs. Was not the kind of guy who went to doctors. And had smoked a lot of cigarettes, 60-pack years.

When he came to the hospital he had a temperature of 101 – 100.1, respiratory rate of 28. He weighed 113 pounds and his SAT was 91%. The white count was 4,400.

And here is his chest CT. And as you can see, there are bilateral pneumonias and there's a cavity in the right upper lobe.

Sputum showed moderate polys and light respiratory flora, and we admitted him to an isolation room.

So the question I'd like to pose for you is what do you do next? What would you do next? And I'll say that there's no one right answer among these choices, but there are some wrong answers.

So I won't necessarily read them out to you, but I'd love to see how the polling goes and then I'll give you my comments on what may be appropriate therapy.

Well, it looks like a lot of you think this fellow has TB and would like to start four drug therapy. I see 75% of you think that that's an excellent answer. And some of you think this could be a different respiratory tract infection. I see about 7% of the votes for giving him broad spectrum therapy with community-acquired pneumonia therapy ceftriaxone and azithromycin plus vancomycin to treat for the possibility of MRSA.

And so I'll go on and say I think that pretty much all of these would be reasonable things to do on admission pending further evaluation of his sputum except for answer D. I think we've learned over the last 20 years that fluoroquinolone monotherapy should be used very cautiously when tuberculosis is on the differential diagnosis. A fellow ID fellow of ours, Dr. Amy Ginsberg, back a few years ago looked at 55 patients who were admitted to Johns Hopkins Hospital over a four-year period who were diagnosed with TB, and among the 35% of them who had been treated with a quinolone prior to their TB diagnosis, 11% of them resulted in quinolone-resistant TB, and that did not happen in the other 65% of patients who had not been exposed to a quinolone.

So in general infectious diseases, we need to continue to educate our practitioners whenever there's a question of TB on a differential, avoid fluoroquinolone monotherapy. We'll come back and talk about this a little bit later in my presentation.

Well, back to quinolones for TB. As the quinolones were being developed in the 1990s, it became clear that they all had activity but that the third and fourth generation agents like sparfloxacin and moxyfloxacin had the lowest MICs, that is the highest activity against *M*. tuberculosis. And those later-generation fluoroquinolones, as the color scheme shows in the PK-PD slide, were also the best – they had the bet pharmacologic parameters with high C-maxes, three to four micrograms per ML, and high AUCs. So it became clear that the leading agents for TB, moxi and gati, had both low MICs and good pharmacologic parameters.

So back in 99, some of my students and Dick Chaisson and I were privileged to get a grant to try this out in mice, and moxifloxacin alone, as well as moxi plus INH prevented mice from dying of TB. The reviews came back with English that sounded like a French guy had written it, and indeed it turned out to be Jacques Grosset who reviewed this paper. And Jacques, of course, has done much of the seminal work already on quinolones for TB.

I'll show you just some of it in this next slide. Looking across the board at different regimens, the yellow curve is killing of *M*. TB in mice over four weeks, and you can see that INH does, indeed, kill very well, with about two logs of killing. That is a hundredfold killing. And so does moxifloxacin at a relatively low dose, 100 milligrams per kilogram, moxi has fine killing potential, superior to that of sparfloxacin.

So this got us all pretty excited about trying to put the quinolones to work in TB, and we began to contemplate studying quinolones in combinations in mice to see how they might fit in with multi-drug therapy. And just to remind you, the killing of *M.* TB in a mouse is in two phases. There's a quick kill and then there's a slow sterilization. And the different antibiotics have differential abilities in these two phases. Streptomycin and INH are the most famous killers, bactericidal agents, whereas pyrazinamide and rifampin seem to do the job with sterilization. And we'll come back to this paradigm later on. It's also possible with mice to treat them and then stop treatment, and then wait about three months and see if any of the mice relapse. So in mice you can do essentially a Phase Three study of treatment regimens and beyond that, what you can't do in humans but you can do in mice, is to sacrifice them along the way, take out their lungs and count bacteria to see how the antibiotic regimens are doing.

With that in mind, we set out with the assistance of a then very gifted infectious disease Fellow, Eric Nuermberger, to try quinolones out in the TB mouse model. And I think you all are aware of the abbreviation system. Isoniazid is H. Rifampin is R. Pyrazinamide is Z. And this, of course, is standard therapy shown in the purple curve. And you see that biphasic kill, with the plateauing out in late phase, essentially the continuation phase, and you see that untreated mice continue to have a high burden tuberculosis.

So the first thing that Eric and Jacques and I tried was the knee-jerk thought, let's add moxifloxacin to standard therapy. Now you notice that ethambutol is missing, and that's because ethambutol is unnecessary when we treat drug-susceptible TB. It's a safety drug in case patients turn out to have MDR or XDR, so we left out the ethambutol in this experiment, but I'm quite confident that it wouldn't have made much of a difference.

So you see that when we added moxi it was a bit of a disappointment. We got slightly better killing in this red curve but not by a lot. And that was a sort of a disappointment. But then a surprise came. When we took away the isoniazid and put moxifloxacin in its place, 2MRZ, 4MR, we got this blue curve where a massive amount of killing occurred in the first two months. There was only log of bacteria in the lungs. One log is ten bacteria, on average, and by three months there were zero CFU counts. So taking away the isoniazid and putting moxi in place seemed to have exciting potential in the mouse model. This was in 2004, and when the clinical trial population saw this, they got excited. And they said let's test some of these regimens in humans.

Before I get to the clinical experience I just want to say a word about why substituting might have been so good rather than adding moxifloxacin. And here is a wonderful experiment done by Jacques Grosset and Deepak Almeida where they did just a very simple thing. First, they treated mice with isoniazid alone at different concentrations over eight weeks. And you see that the lung counts of bacteria, even with a very low dose of isoniazid, go down just a little bit. One milligram per kilogram. When they gave three milligrams per kilogram they got better killing. Higher doses, better killing. Still higher doses, even better killing. Twenty-five milligrams per kilogram even better killing. And all the way up to 50 milligrams per kilogram, very good killing. So isoniazid gave a nice dose-dependent response in killing.

Then they took a different base regimen, rifampin-pyrazinamide, which also gives very good killing over eight weeks, and they added back those very same amounts of isoniazid. So here you see what happens with a base of rifampin-pyrazinamide where we've added isoniazid at 1.56 milligrams per kilogram. Surprisingly, adding isoniazid made the outcome worse. Following the pattern of the last experiment, when they added a higher dose, 3.13, killing was yet worse. When they added more, killing got worse. All the way up, as you can see in this progression, the more isoniazid, the worse the killing.

So in contrast to so many things in life, when mice are receiving rifampin-pyrazinamide, adding more isoniazid is paradoxically not better. More is not better. And this underscores the fact that there is an antagonism between isoniazid and the combination of rifampin-pyrazinamide. Indeed, it's been said many times that perhaps we've been shooting ourselves in the foot with standard therapy making it less efficient by combining isoniazid with the antagonistic combination of rifampin-pyrazinamide.

This begins to explain the mouse study that I talked about earlier where substituting out INH and replacing it with another cidal drug like moxifloxacin could do so well. First of all, it relieved the antagonism of H and RZ, and then second of all, we were adding a very good killing drug, moxifloxacin.

So this was the basis for beginning to contemplate these mouse-derived combinations in humans.

There were a total of four Phase Two serial sputum culture conversion studies where patients were followed on treatment for eight weeks, and their sputum is taken, and the end point is a culture conversion from a culture positive to culture negative. And I won't go through all of them, but I'll take you through the Burman, Chaisson study where moxifloxacin was added to standard therapy, or, stated another way, it was used to replace ethambutol so that HRZ was still there as well as HR. And then later where the substitution that I spoke so much about was tested by Dorman, et al.

And let's see if the mouse regimen predicted what would happen in humans. Can we trust our mouse models? Here is the hound dog leading the hunters, and the hound is saying to himself, "I can't smell a damn thing." Well, let's see if our animal models were doing us justice.

So to remind you, when we simply added moxi to standard therapy, or stated another way, when we put moxi in a place where ethambutol would normally be, we got a small effect. So we would predict that doing that in humans would give a small incremental effect. And that was, in fact, what was done in Study 27, and as you can see it involved about 240 patients with a daily regimen and also a thrice weekly regimen. And these patients were monitored for eight weeks. And the outcome was sputum culture conversion. Of course, that's different from cure. Cure is a Phase Three study, and we'll come to that shortly, but these are surrogate end points looking at only eight weeks.

So here you see the results of Study 27. With two weeks and eight weeks, the moxifloxacin regimen where moxi was added in place of ethambutol and just the standard therapy regimen really were no different. But there was an effect during the eight weeks where moxifloxacin led to higher rates of culture conversion at four weeks and then at six weeks, and that that was statistically significant.

So this essentially exonerated the mice. This is pretty much what the mouse model showed. No big excitement by putting moxifloxacin in place of ethambutol.

Let's see about the other sample regimen where we replace isoniazid with moxifloxacin. This is what the mouse model would predict to be highly beneficial. What happens when we test humans between zero and two months in a Phase Two serial sputum culture conversion study, and that was done in Study 28.

Here you see it was the same design. Patients randomized for eight weeks to receive either standard therapy or moxifloxacin in place of isoniazid.

Well, this study was done in 344 patients at 26 sites. Many of the patients actually came from North America and others from Uganda, and the patients were well matched. And here you see the results. Whether it was in solid media or liquid media, and I'll call your attention to the solid media results, the black dots being standard therapy, shown here, and the gray dots being moxifloxacin, there was really no difference in the rate of culture conversion between the two regimens. That was also true whether one looked in liquid media. No difference between standard therapy and swapping out isoniazid and putting moxifloxacin in place.

So here, in contrast to the excitement in mice, moxifloxacin, in place of isoniazid, was pretty much the same as standard therapy. And this was published in 2009.

So the Phase Two studies paved the way for confidence that at least moxifloxacin-containing regimens were not hurting patients, and they seemed to be equivalent, at least at the two-month mark. And this stimulated enthusiasm for ponying up considerable amounts of money to do a Phase Three study where test regimens would be used for the full six months or four months, and then patients would be monitored

for at least 12, sometimes 24, months to see how many of them relapsed. And the end points, of course, in these studies were treatment failure at the end of treatment, four or six months, and then relapse at the end of the follow-up period. And as I mentioned, these are, of course, much more extensive than the Phase Two studies.

So late 2014, three of these were co-published in the same issue of the New England Journal of Medicine. The first one was the OFLOTUB study. Where as you can see a fluoroquinolone, namely gatifloxacin, was used in place of ethambutol. A very simple design. And treatment was shortened from six months down to four months.

The second study was the REMox study, which had three arms, standard therapy, and a quinolone was used in place of ethambutol, just like the previous study, and treatment was stopped at four months and the patients were followed. This study also did the substitution analysis where a quinolone, namely moxi, was used in place of isoniazid, and that was the third arm.

And then the last of the three studies was the RIFAQUIN study which, as you can see, had a single four-month study were moxifloxacin was used in place of isoniazid. And then in the continuation phase, moxifloxacin continued to replace isoniazid but the rifamycin was changed from rifampin-R to Rifapentine-P. And then another regimen, a third regimen, was tested in which the continuation phase was a standard four months, but it was a highly intermittent four-month regimen with treatment given only one day a week with moxifloxacin and a high dose of Rifapentine, 1,200 milligrams.

So let's start at the first one, the OFLOTUB study. The first of three. And I'll take you through the results that were published in the New England Journal.

This study involved 1,800-plus patients. Twelve hundred completed the study in a per protocol analysis. The patients were followed for a full 24 months, and it was done in five sub-Saharan countries with about an 18% rate of HIV co-infection. And some of the patients were allowed to be started on ART during the treatment phase, but this was relatively rare in this large study.

So here are the results. An unfavorable response was either treatment failure or relapse. And as you can see, with standard therapy, an unfavorable response occurred 11% of the time in a per protocol analysis. And with the four-month regimen, with gatifloxacin in place of ethambutol, and then continuing gatifloxacin into the continuation phase, the unfavorable response rate was almost 18%. This was worse than standard therapy in a statistically significant way. Or stated statistically correctly, this experimental regimen failed to show non-inferiority. The goal was to show both regimens were the same, that is the new regimen was non-inferior to standard therapy, but the statistics showed that yes, this new regimen was inferior. Non-inferiority was not demonstrated.

So why did this happen? Well, the issue was relapse. That was the major reason for unfavorable outcomes. There were relatively rare treatment failures at the end of either four or six months. One important thing that was observed was no adverse effects of the fluoroquinolones, and particularly with gatifloxacin there was some concern that there would be some dysglycemia, but that was not seen.

And also there was quite a bit of variability between sites. The unfavorable outcome rate in South Africa was almost 26%, whereas in Guinea it was only 13%. There was a skew in HIV positivity. Almost half the South African patients were HIV positive. Only one percent of the Senegalese patients.

And there was also a skew with respect to cavitary TB. In two of the countries, South Africa and Senegal, 90% of the patients had a cavity, whereas in three of the other countries only 20% had a cavity.

Here you see that when the subgroups were evaluated there were some interesting subgroup analyses pearls. Looking geographically, with this dotted line differentiating whether the gatifloxacin-containing regimen was better versus control being better, you can see that in Senegal and in South Africa, the

gatifloxacin four-month regimen was significantly worse than standard therapy with these error bars not even crossing the zero line.

And similarly, with respect to patient risk factors, there were some other interesting observations. In individuals who had a low body weight, a BMI of 16 or less, the gatifloxacin-containing regimen was as good as standard therapy. And in patients who lacked cavitary disease, the gatifloxacin regimen was as good as standard therapy. Of course, the subgroups didn't have enough power to make statistically-significant statements, but they were interesting food for thought that perhaps in underweight individuals the regimen could be of some interest. And when there was non-cavitary disease, the gatifloxacin regimen would be of some interest.

Just taken from the authors' comments, why did the study fail to show lack of non-inferiority. Well, they said the patient population was highly varied. And they also pointed out that instead of the old fashioned emphasis on treatment failure – that is I should have said relapse alone, this was a composite end point. And they did that because that's what the regulators wanted. So in addition to relapse or treatment failure, the outcome was also dependent on death and dropout.

The authors dismissed medication variability, lab variability, or the open label design, and I agree with that, that those are unlikely to explain the failure.

Let's go the second of the three studies, namely the REMox study which, as I mentioned, looked at substituting a quinolone for ethambutol and then running it out for the full continuation phase, as shown in the middle bar, or actually substituting a quinolone in place of isoniazid. MRZE for two months, and then MR for two months.

Well, this study was similarly large, 1,900-plus patients, 1,548 completed the study. There was a shorter follow up, namely 12 months. It was done in a larger catchment area, nine countries from three continents, South Africa contributing the largest number of patients at 47%. And there was a seven percent rate of HIV co-infection in the TB population.

And here were the results for the REMox study. Standard therapy had an unfavorable response, that is a relapse or treatment failure of eight percent. The moxifloxacin in place of ethambutol had a 15% unfavorable response which was statistically worse than control. And substituting moxi for isoniazid had a 20% unfavorable response, again statistically demonstrating lack of non-inferiority.

What were some of the observations from this study? Again, relapse was the culprit. Treatment failure was not an issue at the end of four or six months. Importantly there were no significant fluoroquinolone adverse events. With moxifloxacin we have concerns about joint arthropathies and Achilles tendonitis and QT prolongation, and neither of those were detected.

The study was heavily influenced by the South African arm. One very interesting assessment by these authors was a nice discussion that, to put an old issue to rest, when they carefully looked at the patients from Asia, which were mostly Indian patients, and those from Africa, there was no difference at all in the outcomes between these two regions. It had long been said that TB in India was more indolent and TB in Africa was more aggressive. That did not prove true in this multinational study.

And interestingly, using quinolone in place of ethambutol seemed slightly better with a failure rate of 15% versus putting quinolone in place of INH, which had a 20% failure rate. Then the author said well, the obvious reason is we were using three drugs in the quinolone-for-ethambutol regimen whereas the substitution for isoniazid, only two drugs were being used in the continuation phase.

Here you see that the study was dominated by South African subjects, 47% out of the 1,931. And you can also see that the regimens performed very well during the treatment time. So here is 26 weeks, the six month mark, there were really no differences in treatment failures, but the problem came out towards the

end of the study, a year after treatment completion, where the two test regimens in red dashes and green dots show statistically worse relapse rates.

One interesting note that the authors also pointed out was that with the new experiment or regimens that contain moxifloxacin, there was a slight advantage in culture conversion. In fact, they essentially exonerated the Phase Two studies. Here you can see at eight weeks, the transition from intensive phase to continuation phase, there was an advantage in the moxifloxacin groups as opposed to standard therapy in blue, and that was true whether it was solid media or liquid media.

So just to paraphrase what the authors said the reasons for failure were, they did a little mouse bashing. They questioned the reliability of the mouse model. They questioned the predictive reliability of the Phase Two studies, they were called into question, and they pointed out that we certainly need improved biomarkers. And going forward we need a way to do faster and cheaper Phase Three studies. It's generally believed that these Phase Three studies cost in the neighborhood of $20 million.

So the last study, the RIFAQUIN study, number three of three, had a very interesting design. It replaced INH in both of the test arms, and in the second test arm it used rifamycin in place of rifampin at 900 milligrams, or 15 milligrams per kilogram, with a twice weekly design. So essentially they were trying to do away with isoniazid, and shorten treatment, and increase the intermittency.

And then the third regimen was a highly intermittent continuation phase with moxi and Rifapentine at very high dose, 20 milligrams per kilogram, given once weekly.

And, of course, as I think you know, Rifapentine has a much longer half life than rifampin, ten to 15 hours as opposed to two to three hours, and it gives a much higher AUC, more exposure to the rifamycin than Rifapentine.

So in the RIFAQUIN study, a smaller number of patients this time, 827. Five hundred fourteen completed therapy. These were spread across three arms. There was 18 months of follow up time, and this was done in four sub-Saharan countries and had a 28% incidence of HIV co-infection.

And here you see the results for the RIFAQUIN study. Standard therapy gave an unfavorable response of 4.9%. The four month regimen replacing isoniazid with moxifloxacin gave an 18% unfavorable response. This was highly statistically significant in showing lack of non-inferiority. But interestingly, the highly intermittent regimen, Regimen Three, gave an unfavorable response rate of 3.2%, and was, therefore, as effective as standard therapy.

Observations from this study were that, again, relapse, not treatment failure, was the major reason for unfavorable outcome. No quinolone adverse events of significance were noticed. The authors pointed out that with adherence in the weekly and biweekly continuation phase regimens was higher than with standard therapy. And they pointed out that the biweekly Rifapentine at a lower dose gives a higher weekly AUC than the high dose, and therefore, since the once weekly lower AUC regimen did so well, maybe AUC is a little too much – too heavily emphasized, and Cmax may be an important parameter with the rifamycins.

But here is a very interesting observation, specifically adherence. The adherence was about the same, 100% in blue, 95% in red, etc. About the same in the first two months of the intensive phase. But the twice weekly, four month regimen had very high rates of 100% compliance, and the once weekly, six month regimen similarly had very high rates of adherence. So this was a very valuable observation, and I'll point out that while all patients received 56 doses in the intensive phase, in that six-month regimen, the one that was as good as standard therapy, those individuals only had to take 18 doses after they finished their first two months of treatment. So it's a highly intermittent regimen which would, obviously, reduce directly-observed therapy costs if used more widely.

Here you see, again, the reason was relapse at six months or four months, the numbers of treatment failures were about the same, but the problem was relapse of the four-month regimen out at the one-year follow up. Or 18 months after treatment enrollment.

So what did the authors have to say about their study? They bragged about the fact that it had a higher rate of adherence, as they well should have, with the once weekly continuation phase. They pointed out that it doesn't require isoniazid. That third regimen of six months with once weekly continuation phase, is an INH-free regimen. Good if directly observed therapy is mandatory. It would clearly decrease the cost of supervision. One issue, though, is that Rifapentine needs to be given with food, and in this study, every Rifapentine dose was given with two boiled eggs and bread for every subject. And then, of course, another issue is cost with moxifloxacin and Rifapentine being relatively expensive.

I'd like to just very briefly touch on a fourth study, one done in Chennai and published in 2013, where they added gatifloxacin or moxifloxacin in place of ethambutol. So similar to the OFLOTUB study. And this also used some intermittency. The two test regimens with gati in place of ethambutol and then continued, or moxi in place of ethambutol and then continued, were both thrice weekly studies.

This study was stopped by the DSNB prematurely after only partial enrollment because of inferiority of the test regimens and too many failures at the end of treatment. But one interesting note was that the moxifloxacin regimen, while much worse at two months of enrollment, began to be comparable to control treatment way out at two years after enrollment. This failed to showed statistical significance. The rate of recurrence was six percent in standard therapy and ten percent in the moxi arm. It was fully 16% in the gati arm. But this, again, pointed out that perhaps there's something to moxifloxacin and maybe there's further work that could be done with using intermittent moxi-containing regimens such as we observed in the RIFAQUIN study.

So to take a breath and summarize the outcomes of these three Phase Three studies, all three were set up as non-inferiority trials versus standard therapy. And in each there was a four month quinolone-containing regimen, and in each that four-month regimen was not non-inferior to standard therapy. That is, standard therapy was better each time. But RIFAQUIN showed that a six-month regimen was as effective, and that's a cause for some notice. The unfavorable outcomes were always due to a relapse, not treatment failure. And very importantly, there were no significant adverse effects of quinolones in long-term use.

So why did this happen? Why did we spend so much money to get three negative results with four-month studies? Well, I think that it comes back to the difference between sterilization and bactericidal activity. Culture conversion at eight weeks is a measure of how well the regimen kills. And relapse after completing therapy and waiting a year or more is a measure of how sterilizing the regimen is. And just to remind you of what I was saying earlier, culture conversion is a measure of bactericidal activity that we can observe in the mouse model. Sterilizing activity is harder to measure in humans. You have to finish the trial and then monitor them for relapse.

Well what do we know from the mouse model about these parameters for quinolones? I showed you this slide before. Moxi has excellent killing activity, perhaps even better than isoniazid. So moxi is a very good bactericidal drug, and that was borne out in the Phase Two sputum culture conversion studies. But where moxi falls short, and this is probably true of all the quinolones, is in its ability to sterilize. And here is a very interesting study by Dr. Grosset, who gave my standard therapy for eight weeks and then gave them only isoniazid in purple and only moxifloxacin in red. And then standard therapy would be in green. With standard therapy, good killing and auto sterilization. But with moxi alone, the sterilizing activity was inferior to that of isoniazid, so quinolones are not as powerful as sterilizing drugs as even drugs like INH which are considered poor sterilizing drugs.

So the sterilization issue is the problem. Moxi, when given alone, is not a good sterilizing agent. But for bactericidal activity, moxi does well. And perhaps that explains these Phase Three studies and perhaps

the answer was staring us in the face in the mouse models before we invested so heavily in the Phase Three studies.

So I want to pause and do another case before going on to the final two smaller portions of the talk. Here is a woman, 80 years old, who is from suburban Maryland, who was referred to me by a rheumatologist. She had developed progressive left hand stiffness, then bilateral shoulder and knee pain. She went on nonsteroidals as well as pulse dose steroids but they were ineffective. And the infectious disease consult was to ask the question is it safe to use an anti-TNF agent?

Well, the story was more complicated. At the age of 21, back in 1955, she had had pulmonary TB. This was early on in the antimicrobial era. She got nine months of rest therapy in a sanatorium, and then isoniazid, streptomycin, and PAS. That was a failure. She had to go on to a right upper lobectomy, and that did the trick. She improved, went off antibiotics, married, had four children, and was relatively healthy except for some other medical conditions. No more tuberculosis.

Unfortunately her records were discarded. We had no idea of whether this was a drug resistant strain that was associated with failure. Her current meds were ibuprofen and Norvasc. She had normal counts and LFTs, and her tuberculin skin test was three millimeters.

So the question for you is, we're going ahead with the TNF inhibitor. Should this woman receive further TB therapy with the intention of giving her a TNF inhibitor? The choices are yes, give more TB therapy. B, no. And C, maybe.

Well, I see some rapid voting accumulating, and it looks like about 70 of you have answered, and about 70% say yes, we should give this woman more TB therapy. But 15% of you say no or maybe.

Well, my second question for you is this. Let's say we are going to give her more therapy, and we're going to treat her for latent TB, treatment for latent infection. What regimen should we use? And I agree that she should get some treatment. That would be my answer to the previous question. But now the question is what to give her. Should it be INH for nine months, INH for six months, rifampin for four months, or rifampin PZA for two months?

It looks like the votes are coming in, and I see that almost 80% of you are voting for regimen C, and I would totally agree with you. I think there's only one wrong answer, and that would be answer D. rifampin PZA has been recommended against by CDC because when it was studied a decade-and-a-half ago it had some unexplained deaths in patients with latent infection. But INH for nine months and rifampin for four months are recommended in the guidelines. But the issue here is that this woman was already treated with isoniazid back in the fifties, and she may have an isoniazid-resistant strain. So I think for her latent TB infection, if we are going to give her anything, it should be rifampin for four months.

So very briefly in the remaining 15 minutes or so I'd like to talk about quinolones and experimental regimens and then talk briefly about some special situations.

What about rifamycin-quinolone combinations? The success of the RIFAQUIN study got everybody thinking about using Rifapentine with quinolones, and there's some exciting data from the mice that that has promise. And here you can see that Rifapentine gives very high AUC levels because of its long half life where as rifampin always drops below the MIC of the organism when used daily.

So going back to the mouse model, I'm going to show you some data about treatment – bactericidal activity of Rifapentine, moxifloxacin and pyrazinamide, but also show you the sterilizing potential. And here you see that with two months of treatment, Rifapentine, 50 mgs per kg, moxifloxacin and pyrazinamide will kill almost all of the organisms, but that that regimen and all of its partners are not sterilizing. All of the mice show relapses.

But if we go out to a total of three months, Rifapentine, moxi and pyrazinamide not only kill all the bacteria – no bacteria is appreciated in any of the mice at three months, and then we monitor them for relapse, we see that in this regimen, Rifapentine, ten mgs per kilogram, moxi and pyrazinamide five days out of seven, there was not only complete killing, but also lack of relapse. So studies like this have prompted a lot of excitement about combinations of Rifapentine and moxifloxacin. And here you see that with Rifapentine, ten mgs per kg, moxifloxacin and pyrazinamide five days out of seven, at ten weeks of therapy we can prevent relapse and cure all the mice.

So most studies have showed some promise of combinations of Rifapentine, moxifloxacin and pyrazinamide. This is probably due to the increased rifamycin exposure because of the long half life of Rifapentine. And Rifapentine can be used more than just weekly, and that was also shown in the RIFAQUIN study.

So I think we should stay tuned for more work on more use of Rifapentine, and indeed the Tuberculosis Trials Consortium is looking at moxifloxacin, Rifapentine and pyrazinamide combination regimens looking at two days a week and three days a week, increasing the exposure of Rifapentine in the setting of moxi and pyrazinamide.

Lastly I'd like to talk about a few special situations. I'll start with a case. This is a two-year-old child in Baltimore who has fever, malaise, and has fallen off of his growth curve. He visited his grandparents in India for seven months and now has a 12 millimeter tuberculin skin test. The chest x-ray shows bilateral patchy infiltrates at both bases, and another doctor started him on standard therapy HRZE.

You see him now after being on treatment for eight weeks, and his gastric aspirates times three are No Grow So Far. Both of his parents have positive IGRAs but negative chest x-rays. The child is still intermittently febrile, and the chest x-ray is being read as worsening.

So the question is, what would you do, and please vote. Would you stay the course with four-drug therapy narrowing down to a two-drug continuation phase? Would you ask the micro lab to hold the cultures until 12 weeks? Would you request a bronchoscopy in the operating room? Do you think we should expose this child to x-rays with a chest CT? Should we switch to an MDR cocktail that includes a quinolone? Or should we switch to an MDR cocktail that excludes a fluoroquinolone because of concerns of quinolones in children. And I'll add that there is, in my view, no one right answer for this case.

So it looks like a lot of you are saying that we should – about 55% are saying that we should do a bronchoscopy, and about 40% of you are saying that we should add an MDR cocktail that includes a fluoroquinolone. I think that's very interesting. I discussed this case with a Peds ID colleague of mine, and he pointed out one thing which, of course, is that a bilateral patchy infiltrates in a child are not typical in tuberculosis. In fact, the most important hallmark is hilar adenopathy. And we don't know if this child has hilar adenopathy. It's often missed on a chest x-ray. So I think a chest CT to look for hilar adenopathy is essential. And I think we're always going to be on unstable ground unless we make a heroic try to get the organism. So I would both get a chest CT and do a bronchoscopy in the operating room. It's also certainly within the realm of possibility that this is not TB, and a bronchoscopy would help. It might reveal a different pathogen, and that might account for this child's treatment failure.

So I would not switch him to an MDR cocktail just yet. I would probably keep him on TB drugs but try to do a more intensive workup with a CT and a bronchoscopy.

This is also a case that gives me a chance to review the literature of whether quinolones are safe in children. And I'll point out that only Cipro is FDA approved for use in children. The others are theoretically contraindicated. However there's growing use of ofloxacin and moxifloxacin in children with MDR TB. And a recent review in Thorax showed that children with MDR TB, 137 of them, almost all of those children, 96.4%, received ofloxacin. Another 1-1/2% received moxifloxacin. Now this study came from South Africa. So the South Africans in MDR TB in children are going straight for the fluoroquinolones. And it's important to recognize that the pediatric infectious disease community has come out in 2011 with actual guidelines

saying that the experience with quinolones in children has failed to replicate the arthropathy noted in beagle puppies. There's a general consensus that the benefits of quinolones outweigh any risk in drug-resistant tuberculosis. So I think we, when needed, in children quinolones should be used without too much worry.

Another case to get us thinking about special situations. This is a 67-year-old diabetic man in Baltimore. He emigrated from Afghanistan two years ago. He comes in with cough, fever and weight loss for three months. Two months ago he was treated for community-acquired pneumonia with levofloxacin for a ten-day course. Now he's AFB smear positive and was started on four drugs. And the culture turned positive at three weeks. And here is his chest x-ray. You can see patchy infiltrates on both sides, right greater than the left, and what looks like a cavity at the right apex.

At six weeks the lab calls with the following drug susceptibility test. There is resistance to isoniazid and susceptibility to Rif, ethambutol and streptomycin. The question for you is what to do next? Stay the course with standard therapy? Drop the isoniazid but continue Rifampin, pyrazinamide, ethambutol for six months. Drop the isoniazid but continue Rifampin, pyrazinamide, ethambutol for nine months. Ask the micro lab to perform high and low-level INH susceptibilities. Ask the micro lab to perform quinolone susceptibilities. Drop the INH, add moxi and continue RZE for six months. Drop the INH, add moxi, and continue RZE for a total of nine months.

So I can see your votes are coming in, and I'll add, again, that there is no one right answer. I don't know if the system will allow you to vote more than once. I think there are multiple correct answers here.

So it looks like many of you are saying ask the micro lab to perform high and low-level INH susceptibilities, about 25% of you say that. Ask the micro lab to perform quinolone susceptibilities, about 21% of you are saying that. And I would totally agree that those two items make a lot of sense. We need to know if this is low-level INH resistance because there is some thought that one can treat through that. And we would like to know if we can use quinolones for this individual.

I think we certainly need to not stay the course. Well, we need to know the INH susceptibilities before we can make a decision on whether to drop the INH, so I think A, B and C really can't be decided upon until we get the drug susceptibilities. But the classic teaching is that in individuals with isoniazid mono resistance, Rifampin, pyrazinamide, ethambutol are effective.

I would say that answer B is incorrect because this patient has a large cavity and we know that in individuals with extensive cavitary TB, treatment should be run for a total of nine months, so this gentleman is likely destined for a total of nine months of treatment.

But then the question is, should we be using moxifloxacin in INH-resistant tuberculosis? And that's a burning question. And I'll say that there are very little data to support using moxifloxacin in INH-mono-resistant disease with the possible exception of the RIFAQUIN study which I showed you where a six-month course that lacked isoniazid was as effective as control in the third treatment arm. The six-month therapy with moxifloxacin in place of isoniazid and Rifapentine as the rifamycin. That's not exactly what this patient is receiving, so I have to say that there is weak evidence that moxi should be added in place of INH when there's isoniazid mono-resistant disease, but it's a question that certainly needs more research.

I'll also point out that the quinolones come with some hazard. While they're generally well tolerated with mild problems of nausea, headache and insomnia, the tendonitis can really be severe when it happens and lead to significant morbidity. The issue with QT prolongation needs to be taken into account, particularly with an elderly individual who may have some underlying heart disease. And the quinolones have been closely associated with c. diff-associated diarrhea.

So I think we need to be cautious about reaching for the quinolones willy-nilly, and I think we certainly need more research on this question of whether to use them in INH mono-resistant TB, which, as I mentioned, is now ten percent of the tuberculosis in the United States.

I'll also point out that current studies show that quinolone resistance, while on the rise, is still in the low single digits. The highest reports I've seen are below four percent. But we don't want that situation to get worse.

I will also point out, as we mentioned earlier, that quinolones, when used in patients who could have TB, have been clearly shown not only to lead to quinolone-resistant TB, as we discussed earlier, but to lead to a delay in the diagnosis for pulmonary TB. This has been quantified in some recent studies when (inaudible) quinolones are used for what's thought to be community-acquired pneumonia, there's an average of 19 days in the delay of diagnosing the pulmonary TB. And similarly, there is a correlation with mono-resistant, with the development of quinolone-resistant TB, a 2.7-fold higher risk of quinolone mono-resistant TB in patients who received quinolone for what's thought to be community-acquired pneumonia.

So we're nearing the end, and I thank you for your patience. Back to our score card. We started this journey out looking for shorter, more intermittent INH-lacking regimens. I would say that the Phase Three studies have not demonstrated that we can shorten therapy with quinolones to four months. However, the success of the RIFAQUIN regimen shows that quinolones can be used in situations that can lead to much greater intermittency as in that RIFAQUIN regimen.

And similarly, we can eliminate the use of isoniazid with regimens like the one in the RIFAQUIN study.

So to wrap things up, it's finally over. I entitled this study Wounded But Not Dead. Is there a bright side to these three largely negative Phase Three clinical trials? Is the glass half full or half empty? I think we did get one positive result, the RIFAQUIN study, which showed equivalence to standard therapy. And we got some other information that it's worth pointing out that the safety issues of using quinolones for four to six months has largely been put to rest. There's no evidence of QT prolongation, tendonitis, dysglycemia, or hepatitis. Hepatitis has been a concern because of the problems with rifampin, pyrazinamide and latent TB leading to fatal hepatitis with the use of moxi without INH led to some concerns over that.

Therefore I would say that there was also a hint of benefit in the Chennai study which used thrice weekly moxifloxacin in place of ethambutol. And in the subgroup studies from the OFLOTUB study there was the possibility that individuals who had a low body weight were doing equivalent to standard therapy because of greater gatifloxacin exposure. And perhaps there would be a subgroup of individuals, such as individuals who convert their cultures at two months and have non-cavitary disease. And the optimists out there are already talking about this is a four-month regimen adequate to cure patients and a reassessment of the British Medical Research trials in Singapore, in this particular publication suggested that a four-month regimen with a relapse rate of 5.9% was almost as statistically valuable as a six-month regimen, leading to some speculation that we might be able to find the right subgroups with a quinolone-containing regimen that would be as good as standard therapy with four months.

So glass half empty, glass half full. The pessimists are called the dark cloud, the lemons, rather than the lemonade. Dark cloud and not the silver lining. The question for us is are the quinolones for TB going to be in the half empty or the half full side of the coin. The optimists, of course, are accused of telling lies, damn lies, and relying on statistics or politics.

So stay tuned. I think the quinolones are wounded but not dead, and there's much more to be learned. Thank you.

Well, that was absolutely fantastic, Bill. That was great. I want to personally thank you so much for a great, great talk. And the way you know a great talk is you've got a ton of questions, so I know we're running short on time, Bill, so I going to jump to it, but I wanted to start. You know, a little daring for you

guys to – for you to say at the end about politics, huh? I mean somebody from Johns Hopkins actually running for President. Big move there, huh?

Yeah, just for the audience, David and I were joking about incriminating pictures. And I tell my residents and fellows, be careful what you do on camera because you never know when someone from Johns Hopkins is going to run for President again. It's an exciting election, I will say that.

Well, look, we've got a couple of questions, Bill. I mean, you know, the first question you got is, you know, one of the questions about, you know, when we're looking at the three, you know, the New England Journal of Medicine article looking at the Phase Three studies. The question about relapse and treatment failure. If you could quickly just review the difference in, first of all, the definition, and then more specifically the questions were coming through about was it truly relapse or was it reinfection? Was that looked at? And those that did relapse, did they look at the development of fluoroquinolone resistance?

Excellent questions, and the microbiology and the three papers had been done preliminarily. The RFLPs had not been done in most of the – was undergoing analysis to rule out reinfection with the second string. However, in all patients that relapsed, there was an assessment for drug-resistant relapse. And that generally did not occur – I think there was one case of relapse with a mono resistance to rifampin – I would have to look it up. But there was no issue of relapse with drug resistance to quinolones. And so the regimens prevented the acquisition of drug resistance, but they did not cure.

I mean that's fantastic. Thanks. Let me ask you another question. I mean, one of the things that I find is really fascinating, and something you kept alluding to, was the issue of area under the curve with Rifapentine and moxi versus Cmax. And, you know, it seems to maybe hint at that when it comes to sterilization, that Cmax may be more important than area under the curve. Which brings us to our next point, which is there's a number of studies in the mouse model that suggests higher doses of Rifapentine may be more effective and may be shortening. So do you want to comment on the whole concept maybe that the next time around – because the next question is is where do we go from here. And we know that we're already going from here, so is the next steps maybe using the quinolones with higher doses of Rifapentine more often?

Yeah, I would agree with you, David. You know, determining the parameter that is most effective is arduous work, even with an easy infection like staff or e. coli. It takes literally thousands of mice with different regimens to try to figure out whether the antibiotic is working in a Cmax mechanism or an AUC mechanism.

Quinolones are a classic AUC killers, but it hasn't been closely addressed in tuberculosis. So it might be that we are guilty of extending (inaudible) and staph and other bacteria where we believe – you see is the major parameter. And falsely trying to maximize AUC of Rifapentine when, in fact, it could be Cmax.

So I think we're going to see a lot more pushing the dose of Rifapentine. It was exciting when in the RIFAQUIN study patients tolerated 1,200 milligrams. There was some concern that they would suffer malaise and flu-like symptoms. But that was not the case. These studies were done in overseas populations. Those individuals are frequently thought to be more stoic than the American populations, less likely to report their complaints. So it will be exciting that TBTC is looking at these higher Rifapentine dose studies.

Yeah, I mean I don't want to say nothing, but that last statement about, you know, that us Americans may not be that stoic, is going to kill your Presidential run. I just want to make that point. But you know I just want to make one more thing on that and then we'll go on to the next person, but you know, you made a great point that I always want to remind that TB is not staph or strepto. The double time is so slow. And I think that you're right. I understand the problems with showing it, but I think sometimes we may make the mistake of using bacterial pharmacokinetics and pharmaco dynamics for TB, but just – that's just a comment of mine.

My other comment is being from New York there's plenty of mice that we could use in these studies. So don't worry about the mice.

But the other question that we had, all the way from Greece actually, where obviously your comments about the politics probably won't hit as hard, but they wanted to know about how was the drug tolerated as far as like especially with multi drug resistance that – but with the fluoroquinolones, you know specifically, allergies. Did they see drug allergies, rashes? In our experience we don't see that much with fluoroquinolones. And then lastly, you know, there is these statements now as fluoroquinolones as being reported, granted sporadically, of this very severe peripheral neuropathy that seems almost idiosyncratic but seems to occur and then last longer. Any comments on whether the rashes, allergies, any comments on that and also any other side effects besides the typical tendon?

Yeah, the three New England Journal studies focused on severe side effects, and death. And the severe side effects were limited to a handful of individuals in each arm, across the arms of the studies, in all three studies. So they had very few serious adverse effects. And I agree with your point that a neuropathy or a rash might not have fallen under the serious adverse – they also did passive surveillance, I have to say. They did not go out and do EKGs on these patients looking for QT prolongation, nor did they do spot glucose checks to look for dysglycemia. So it is certainly possible that under a North American scrutiny, you would get a side effect profile that was not as optimistic as the ones that came through with these African, Asian and South American patients.

And, you know, just a – other question coming up about the Rifapentine, and I know this was not necessarily a talk on Rifapentine, but I do – I think we both agree that the combination of the Rifapentine and the quinolones looks very, very promising. I think there's going to be a lot more studies. And the question specifically was for us TB personnel who would be the ones giving the drugs, you know, the question is always with Rifapentine and how it's better absorbed with food. The question, I guess, is that how important is it to give food and Rifapentine and what if you don't. And, I guess, more specifically, is that maybe going to be – maybe is that the point about using the higher dose of Rifapentine, how that may overcome that limitation?

My understanding, David, is that those studies are ongoing, the precise nature of the food requirement and how much you can overcome it by pushing the dose. Of course, if you push the dose, and somebody takes it with food and somebody doesn't, then you run into the possibility of getting an ultra high dose in some individuals and not in others and a higher possibility of side effects. But I do think we're going to need to answer that question.

It was interesting to me that the issue of food requirement was posed as an issue for these relatively poor countries of the need to provide two eggs and some bread for these patients. That was actually a significant issue with complying with the requirements of the study. That the sites often had to go to some trouble to make sure there was food on hand for those individuals. That is much less likely to be an issue with North American patients, but it was an issue in these relatively poor countries, as, of course, is the issue of Rifapentine and moxifloxacin cost.

Yeah, I know, definitely. And I always think it's interesting – I agree with you – that they picked eggs as their fatty food and high cholesterol food to use as the absorbent. I guess, always bringing, you know, the Egg McMuffin overseas.

But the other questions that we're getting asked is, you know, you alluded to it, you know, with all the molecular rapid testing for resistance coming closer and closer to home. You know, right now I think most of us have access to at least the gene expert, but some of the INH and rifampin with the Heinz (sp) test, but that the new Heinz test-plus – not new, but the next generation – probably become more rapid, the question that's being – would you suggest getting routine gyrA mutations, or should we be testing for fluoroquinolone resistance especially in patients who may have had fluoroquinolones in the past?

I think in a patient with any drug resistance on their antibiogram, we're going to want the lab to report out the quinolone resistance based on a growth assay.

I know that at our hospital molecular assays for antimicrobial susceptibility testing are not prime time. They may be cheaper and actually be proven to be better, but we're still going with culture all the way. And, in fact, here quinolones are just part of the first line testing whenever there's a positive growth on a TB culture we get a quinolone susceptibility.

So I do think it's important to determine that. Anybody who has a fever in this country for more than a couple of days is highly likely to see an antibiotic. And often that antibiotic is a quinolone. And sometimes patients can't tell you what antibiotic they took, and that raises the possibility that they're quinolone exposed. So I think we're going to see a lot of changes coming down the pike, and I firmly believe that in 20, 30 years standard microbiology is going to be fading in favor of molecular tests, but I think in 2015 we still need the culture-based quinolone susceptibility.

I think that the only thing I would say just as kind of, you know, just is that you know, right now, if you're going to decide – and I know we're not there yet – but if you're going to decide, you know, you're going to use the four-month regimen, the gyrA may be a way to kind of tell you that it may be okay or not. But I think we both agree that just because you have a gyrA mutation, doesn't necessarily mean you're resistant to all the quinolones. And like you said, I think you'd agree and we're still learning, but there may be certain mutations that may make you choose one quinolone over the other, but this is not ready for prime time, I think you agree.

Totally agree.

So talking about one quinolone versus the other, you know, there's been a lot of talk about the quote/unquote Bangladesh regimen, the new, you know, the nine-month regimen for MDR that's really seen some really nice success overseas. And, you know, in that regimen, as alluded to, Bill, is that they use gatifloxacin, but in this country we don't have access to gatifloxacin. And obviously there's no studies to show this, but what is your feelings about the interchangeability – you kind of alluded to it, and I think the reason I'm pointing it out is that it was very important, I thought you made a great point, about how gati and moxi may be – may be similar in their spectrum and effectiveness. But what do you think? What would be your comments on that?

I think if anything moxi is slightly better. They're comparable. And I didn't show the MIC data in large collections of strains, but there is some evidence that moxi is more potent on a milligram-per-milligram basis. So I would be very comfortable using the Bangladesh regimen with moxi in place of gati, and I think it would only be a little bit better, if anything.

All right, just – just, you know, one more ques – I'm sorry, go ahead.

Oh, just want to point out that in that Chennai study that came out in 2013, the gati was by far the worst 3 month regimen. Moxi was the one that seemed to be slightly more promising. So you could even see a difference in those studies head-to-head in the same patient population of moxi being slightly better than gati.

I agree. I think it's just, you know, as we may be based, just looking at how do we, in certain very, very select patients, maybe utilize that regimen. You know, the question is going to come up.

So just one more comment, and then I want to thank you because we're running over. But, you know, so, and this question is really for my colleague Connie Haley (sp), who both of us share the medical consultation here in the South, I think Connie would agree there is not a week that goes by where we're not getting a call from the community about utilizing fluoroquinolones in somebody who is INH resistant, or using fluoroquinolones to make somebody smear negative faster. And I know you – you've alluded to it, but it's an important summation which is that is there a role in either of those situations, you know, we

see it – I think we just love our fluoroquinolones like you said, is there really any evidence to support the use of fluoroquinolones for either of those situations?

Yeah, the short answer is there's no hard data head to head with the existing standard recommendations do not – like with INH mono resistant, there's no evidence that there's an advantage of using – adding a quinolone as opposed to what's in the recommendations to just treat with rifampin, pyrazinamide, ethambutol. And similarly, bone tuberculosis, quinolones do penetrate bone very well, but we just don't have studies to show that they're better, and I'll come back to the fact that quinolones don't come for free, they do have side effects, particularly in elderly, compromised individuals with potential cardiac disease or c. diff risk, it's not a simple decision. So ideally when we see this in five more years, we'll have some hard data on quinolones and those two special situations, but we lack it right now.

I agree. And actually I lied. I said one more, but we've got a great question here. So Ken is asking based on the PK-PD data, what do you think about, you know, could levofloxacin be used in place of moxi, you know, due to its lower cost to most of our TB programs, and the question of possible less QT prolongations. What do you think about levo for moxi, and, again, on the other hand, the whole issue of the lower half life.

Yeah, I think it's an inferior drug microbiologically. I think moxi is more potent from a milligram-per-milligram basis than levo, and so if I were going to use a quinolone, I'd use moxi. I think the price of moxi is going to come (inaudible) as generics start to come onto the market.

I agree. I mean, you know, things are going to change, and I think it's one of these times where all the moons may align, you know, so as we gain more and more data, you're right, I think that moxi will become more accessible to most of us.

Hey, Bill, I said this to begin with, and you far exceeded it. It was outstanding. Unbelievable presentation. I want to thank you so much, and on behalf of all the audience, we cannot thank you enough for taking the time and sharing with us today. And I also want to make one other statement that I think your talk so well illustrated, that we don't give enough credit to. If you look at most of the work – or a lot of the work that we've done, how much important research is being done throughout the country, throughout the world. In particular I want to give a lot of credit to the CDC's Tuberculosis Trials Consortium which has done so much work and continues to do so much work. And Bill, I want you to please take this home to all of your colleagues. We so appreciate all the work you guys are doing at the Johns Hopkins Center for TB Research because we're learning every day. And thanks to that research, we're able to treat our patients better.

So I want to thank you so, so much today, Bill, for joining us. We really appreciate it.

Great pleasure. Thank you for inviting me.