



Transcript provided by kaisernetwork.org, a free service of the Kaiser Family Foundation¹
[Tip: Click on the binocular icon to search this document]

39th Union World Conference on Lung Health Controversial Topics in MDR-TB Management 10/19/2008

[START RECORDING]

JOSE CAMINERO: – all of you know, the resistance history is very recent, and [inaudible] we are speaking about MDR-TB and XDR-TB. For duration, we have very little evidence regarding the management as in diagnosis, as in treatment possibilities. For the duration, when we are analyzing the different possibilities for diagnosis and treatment, we have a lot of controversial topics and very little evidence.

And, as all of you know, at the moment we have a very important problem with the MDR in the world. We are thinking maybe every year, there are about 500,000 new MDR-TB patients, and at the moment it's a very important concern in the world. For the duration, to address the different possibilities, or what are the different controversial issues in the management of this disease, we consider very important.

For the duration in this symposium, we will address five interesting topics to discuss later. I would like to introduce the first speaker, Senor Marcos Burgos, my friend Marcos Burgos. Marcos Burgos is assistant professor of the University of New Mexico School of Medicine, and medical of the Tuberculosis Program of New Mexico in the Department of Health in New Mexico. Thank you very much. Marcos?

MARCOS BURGOS: Good morning. It is a pleasure to be here for the 39th Union World Conference on Lung Health, and I appreciate the invitation to talk about a very interesting topic, and one that may have implications for the future

epidemic of drug resistance. Today I'm going to be talking about the cost of antibiotic resistance of mycobacterium in tuberculosis. I'm going to give you an introduction, later on I'm going to review some important concepts, and then I'm going to talk about some few important studies, because of the limitation of time, and at the end, I'm going to give you some of our data that may have some implications on this topic.

The emergence of the drug resistance to tuberculosis is threatening global disease control efforts. And as we know, it is now a global emergency. In vitro and animal models suggest that antibiotic resistant bacteria is often associated with reduced competitive fitness when compared with antibiotic susceptible strains, but we all clinicians that treat tuberculosis know that patients that have been treated before also can acquire resistance to transmission. And we have seen MDR patients that have never been treated before, and XDR patients that have been never treated before, and this is one of the reasons of the epidemic of DXR in South Africa.

The capacity of drug resistant strains to transmit between hosts, could be an important determinant of the magnitude of the problem in the future. In the last 10 years, we have had an incredible advance in the understanding of the population genetics of *M. tuberculosis*. We are now able to differentiate reliably between different strains of tuberculosis with genotyping, single nucleotide polymorphisms, and now with genome sequence, which is becoming very cheap.

These techniques are allowing us to elucidate the interaction between hosts, the environment, and factors of transmission dynamics. Understanding the ecology of drug resistant tuberculosis is going to be essential for us to preserve the lifespan of the few antibiotics that we have to treat drug resistance.

Key concepts, *M. tuberculosis* is a clinical organism with a high degree of homology. MTB evolution occurs mostly by mutations and deletions. Mutations arise spontaneously, and at a constant rate. This is one of the reasons that we always use more than one antibiotic to treat tuberculosis. It is only when mutations provide an advantage that mutation bacteria increase in a population.

Now I'm going to present some work that we did some years ago in San Francisco, in collaboration with Chuck Daly [misspelled?] and Peter Small, and what we did is we tracked drug susceptible and drug resistant strains in the population, about 1,800 strains. We calculated the secondary case rate of drug resistant and susceptible strains, and we did it for each genotype. We did it for Isoniazid, Rifampin, Streptomycin, monoresistance, and then INH and the Streptomycin, and then MDR strains. What we found was very interesting.

Isoniazid strains, the secondary case rate was much lower than of those for Streptomycin and Rifampin strains. What this means, 0.29, is that Isoniazid resistant strains are 70-percent less likely to submit the susceptible strains, in

other words. When we looked at MDR-TB over a 10-year period, we didn't find any transmission with the drug resistant tuberculosis in San Francisco. Streptomycin's resistant strains, mono-resistant strains, were almost close to one, the secondary case rate, meaning that they were almost as fit, or the capacity to reproduce in the community was almost similar than susceptible strains.

When we looked at the Rifampin strains, something different was going on. It appeared that the Rifampin strains were able to transmit in the community more than susceptible strains. However, when we looked at this group of the strains, almost 80-percent of these strains came from patients with HIV disease. So we thought this explained the reason why we saw an increase in fitness in Rifampin strains.

Later on, Sebastien Gagneux, in Peter Small's lab, in collaboration with us, did a study looking at those strains from the same study that transmitted, resistant strains that transmitted and didn't transmit in the community in San Francisco. And what we found is that the resistant strains that transmitted in San Francisco were those that had the 315 mutation, or a mutation in the INH promoter, and these strains were always in those two groups, and few strains, only two, were among a group that had no mutations. Notice that there was no transmission among mutations other than the 315.

What we thought this implied was that although it appears that the INH was less transmitted in San Francisco,

there was a group of mutations, or specifically the 315, that was almost as transmissible as drug susceptible disease. This study corroborated findings by Dickman Soligen [misspelled?] and Alexander Pim [misspelled?] and Stewart Cole [misspelled?]. The first study was done in the Netherlands, and they found that the 315 was as transmissible as drug susceptible disease. Alex Pim did an in vitro and in vivo test in mice, and found that the 315 produced catalysts, as well as it was as [inaudible] as drug susceptible strains.

This is another study that is not really related to drug resistance, but it could have implications for the development of drug resistance. Let me explain, this study was done by Tony Tsolaki and Peter Small's group, and this is done by a hundred strains were integrated by microwaves to find the lesions or large sequence polymorphisms, or regions of difference. They found 68 large sequence polymorphisms in 224 genes. What you see here is the structure of tuberculosis from different regions of the world.

These strains from San Francisco came from foreign born patients and patients within the United States, but you were able to divide them by the place of birth into four groups, those born in the Americas, those born in Europe and Africa, and those born in East Asia and the Philippines. What this means is that there are groups that appear to be evolving independently of each other in different regions of the world.

This could have implications where we see in terms of epidemic,

in terms of virulence, transmissibility, and maybe the development of drug resistance, and I will explain more of this later.

Another study by Gutacker in JID in 2006, and Gene Maser [misspelled?], found the population structure by single nucleotide polymorphisms in *M. tuberculosis*. They integrated close to 5,000 strains from around the globe. Most of them were collected in Houston, Texas, the United States. Houston has one of the largest foreign born populations in the United States. What they found was that with 36 single nucleotide polymorphisms, they were able to divide 5,000 strains into nine distinct groups.

And what you're able to see here is that these nine groups present different parts of the world, and different strains have – different numbers of them represent larger numbers in some region. For example, as you can see here in Asia, this strain, or this group of – this lineage is overrepresented in some regions in Southeast Asia and China. India, Americas, this region, this lineage is overrepresented in some parts of Central America, South America, and even the United States. And in Africa, you can see that there are some lineages that are more specific to that side of East Africa.

One of the problems with this study was that these – although they integrated 5,000 strains, all the strains were not represented from all regions of the world, meaning large

part of Africa was missing, as well as some parts of Asia and Latin America.

Another important study done by Gagneux, also from Peter Small's lab, shows that the global population structure and the geographical distribution of *M. tuberculosis*, by looking at large sequence polymorphisms. What they found is that they could now differentiate into six groups, as opposed to Tony Tsolaki's four groups, by integrating approximately 800 strains. Most of the strains came from San Francisco, also where a very large foreign population – and other – they had strains from other collections around the world.

What they were able to do is pinpoint where these patients were born, or from the other collections, the geographic locations of these strains, as in Gene Maser's study, they were able to do the population structure of tuberculosis, and you can see the different groups by different colors here, are represented in different geographic regions of the world. Again, as I said before, it appears that some lineages of tuberculosis are evolving independently of each other in some regions of the world. This might have implications for virulence, transmissibility, and also the development of drug resistance, and may explain what we see with respect to hot spots of drug resistance in some parts of the world, and larger numbers of tuberculosis cases around the world.

Another study by Gagneux, published in Science in 2006, is a very elegant study done in an in vitro model. And what we find here is the competitive costs of antibiotic resistance in *M. tuberculosis*. What they did was look at the relative competitive fitness of laboratory-derived Rifampin-resistant strains. Basically, these study with a susceptible CDC 1551 strain that is very commonly used in laboratories around the world now, and they selected for Rifampin resistance. They also use – this is the light grey bars, the dark grey bars is the one East Asian, or the Beijing strain, and in this susceptible strain, they selected four Rifampin-resistant strains in the laboratory.

What they found is that all of the strains, and here let me explain you have the mean relative fitness, if it is close to one or the fit is one, that means a drug's susceptible and a drug resistant strain has the same fitness, the same capacity to grow. What they found was that in general, all the laboratory derived, the strains, were less fit than the drug susceptible strain, with some important caveats. The one with the 315 mutation in general, tended to be – the fitness tended to be closer to the drug susceptible strain, and this is important because the 315 mutation, and also the 526 mutation, are the most important or the most commonly found mutations in clinical strains. These strains in this area here are the mutations, and these strains are rarely found in clinical

strains, and these were associated with less mutation by the 529 mutation – with less fitness, like the 529 mutation.

Then they did something very elegant as well. They then looked at mutants that were selected clinically, meaning patients that were initially susceptible to drug resistance, but then because of management issues, they developed Rifampin-resistant mutants, and they tested those mutants from different lineages, different strains – they tested them with the same competitive cost analysis in the laboratory. And what they found is that most mutants, all of these mutants here have the 531 mutation, and all of these mutants were more closely had – I'm sorry, they had, it appears, higher fitness than the drug susceptible strain, like this strain here, the number five, which is of the Euro American lineage. You had a higher fitness than the drug susceptible strain.

These mutations here were not commonly found or are not commonly found in clinical strains. What this means is that maybe those that are not selected clinically are going to be less fit and that's why we don't see them in the population as much.

These findings have important implications for maybe the trajectory of drug resistance epidemics in the world, meaning that maybe drug resistant strains, not all of them, have a decrease in fitness or capacity to cause epidemics, and that maybe there are some strains that have equal fitness to a

drug susceptible strain, and that's transmitted in the community, or even higher fitness.

So, in my laboratory, what we wanted to do is investigate the growth of clinical strains in human macrophages to assess the cost of antibiotic resistance. It is very hard to work with human macrophages, so it is very difficult to do competitive fitness. So what we did is we – the same clinical strains derived from patients that developed resistance, and we integrated them for genotyping and found them to be the same genotype with three markers. We grow them in human macrophages from donors between the ages of 25 to 35 years of age. And here we had ten pairs of strains with all of them started with drug susceptible strain, and then they developed resistance to Rifampin, INH, or developed resistance to MDR, and we had the mutations in most of them that explain the mutation in the INH, in the *katG* gene, or the INH resistant mutation, or the Rifampin strain.

We found that the 526 and 531 were the most common mutations in this group of strains like in Gagneux's paper. What we found is that the resistant paired isolates of Isoniazid, the chloroform, in units in macrophages. The drug susceptible strength grow much faster than the drug resistant strain when there was a mutation in the initial [inaudible] of the *katG* gene.

The drug susceptible strain grew much rapidly again here when compared to the H37Rv, and the resistant strain when

he had a mutation in the [inaudible] of katG. And the same here when he had a mutation in one or two. But in here, we have not yet confirmed the mutations, but we didn't find mutations in INH, but what we found was very striking. The INH resistant strain grew much faster, very lots of difference, then the susceptible and the H37 strength. And the findings get more interesting.

What we found then was that the Rifampin strains, those that became resistant to Rifampin in these patients, grew much faster than the susceptible strain and the H37Rv strain. The same with the 526. He grew much faster than the susceptible strain and the H37Rv strain. When we looked at the MDR-TB strains, those that developed resistant to Isoniazid and Rifampin, we found that all of them grew much faster in macrophages than the drug susceptible strain or than H37 strain, and there are different kinetics here that could be explained maybe by the lineages of these strains, and also the mutations of these strains. For example, here you can see the 315, the one that was reported as not having lost fitness, and also the 531 that was reported by Gagneux as having not lost fitness, or gaining fitness, was seen to grow faster in the macrophage model.

So, the summary of the results, all clinical isolates resistant to Rifampin or MDR had increased growth kinetics in macrophages. The rpoB mutations identified were the 531 and the 526, which were the same mutations that Gagneux found to be

almost as fit as the drug susceptible strains in clinical isolates. We found one MDR isolate that had the 315 and three that had no mutations identified in genes associated with Isoniazid resistance. Of the eight paired isolates with increased growth in macrophages, one of them are of the East Asian, and five of seven are the Euro American lineage.

In conclusion, the heterogeneity of growth in human macrophages may be a function of drug resistant mutations and the probability, and also the potential impact of the genetic background of the strain. Compensatory evolution, or increases in fitness within the host evolution may be responsible for these observations. However, the adaptation to growth in the macrophages environment might also account for these findings. That means that maybe they adapt to growth in the macrophages, but that doesn't mean that they're more transmissible in the community.

Our work supports the findings of the in vitro competition assays described by Gagneux and colleagues, for the study in drug resistance ecology and evolution. The findings that fitness changes can occur during infection of a single patient has importance to the tuberculosis control and the prediction of drug resistant epidemics in the future. Thank you very much. [Applause].

JOSE CAMINERO: Thank you very much, Marcos. I think we can postpone the discussion until the end of the symposium.

I would like to introduce now Sang Jae Kim, our friend Sang Jae

Kim. He is laboratory consultant of the Union, and he was the former director of the Korean Institute of Tuberculosis.

SANG JAE KIM: Good morning, everybody. I have been asked to talk about the reliability about the drug susceptibility testing. Well, reliability of drug susceptibility testing is – actually, I may talk on the reliability of conventional drug susceptible testing, rather than rapid cross-based tests or molecular techniques.

So, the current convention of drug susceptibility testing certain suffers from poor predictive barrier of the results and poor test reproducibility.

How well the laboratory identify the phenotypical resistance? It can be identified by the conventional growth based method, or by rapid growth method, or molecular techniques. So this is a laboratory side. But another one is clinical drug resistance. This should be calibrated, keep in mind what kind of the criteria we have to use to identify the best the clinical trial system can isolate. This has to be calibrated with the probably susceptible and probably resistant strains.

So, I will first talk about the laboratory aspect, in other words, how well the laboratory can identify the drug resistance in the laboratory. So, this test of reliability will largely rely on how well you control incorporating accurate concentration of the drug in the medium. So that can vary from drug to drug. The stability will vary from drug to

drug in different mediums, and also the potential calculation and the dissolution and dilution – all this laboratory procedure should be well controlled and standardized, otherwise you cannot expect the desired result. And also, the second most important was how well the laboratory can standardize the inoculum size and also the viability of inoculum, and also the standardizing the test system, and then also incubation, reading, interpreting, and reporting. And, also whether all these tests are performing, and the strict internal and external quality control, also will affect the reliability of the test.

So, I just will briefly touch on the external quality assessment of drug susceptibility test is operating in global drug resistance surveillance network at the moment. So, this is a – I just got this data from Dr. Armand Van Deun, a very recent [inaudible] test result of the 17 supranational different laboratories. So if you – he sent a panel of strains in which 17 are resistant and 13 are susceptible to Isoniazid. As you see here, the average sensitivity, sensitivity, efficiency, reproducibility, is excellent.

So you look at here the 100-percent agreement. [Inaudible] to detect the resistance, 100-percent. Thirteen laboratories showed 100-percent sensitivity. Fourteen laborites 100-percent specificity, which is very good. But few laboratories, if it is less than 95 than this is a real problem. In cases of Rifampicin, again, the outrage

sensitivity, specificity, efficiency, is excellent, all over 98 or 99-percent. Streptomycin is a bit less, and Ethambutol, even worse.

So, I calculated on the basis of his data, so in the case of Rifampicin, the sensitivity, 99.6-percent. Specificity, 97.9-percent – this is excellent. So, taking account the sensitivity and specificity, I calculated the resisted predictive value, so like this. So, again, if prevalence – prevalence is somewhere around 2-3-percent. As you see here, even if such a wonderful [inaudible], still the resistant predictor is somewhere around 50-percent. So this clearly tells you, you cannot try to screen the MDR-TB from the new cases, where the – among whom [inaudible] is very low. If it is less than three, it will be a problem.

So, if it is over 10-percent of the resistant prevalence among either the new cases or the retreatment cases, then it would be okay.

So this is a – I just got this data from the 4th Global Drug Resistance Report, WHO. So this – out of the 51 national or regional reference level laboratories, so approximately 78-percent of those laboratories showed 100-percent agreement. It's not bad. They are doing quite well. And in case of Rifampicin, 73-percent. So between 95 to 99 agreement with six or seven. So if you include these two – so almost 90-percent of the national regional level of reference laboratories show very good approximation of the test to Isoniazid and

Rifampicin. This is resistant predictive value of second-line anti-TB drugs. This – still the data of the second-hand drugs is still not much, so this has been done in 2004 by Nuria Martine in Spain, and she sent a panel of strains to the – I think nine supranational reference laboratories. But at that time, the systems strain in the panel of the panel, resistant to [inaudible] is very few, even Capreomycin, Ofloxacin, is not there, many is strange, so that's why provider sensitivities, all of them are 100-percent. But [inaudible] is very poor. And specificity actually is a program actually. It was [inaudible], Capreomycin, Ofloxacin – Ofloxacin was better, but Calamajin [misspelled?] is not so good.

So, anyway, based on this sensitivity, specificity – if you calculate the resistance predicted value at the – prevalence. So, it should be over – if, in case of second-hand drugs, if prevalence is less than 30-percent, still it wouldn't be a problem. Okay? It's not satisfactory.

This is another report. Also the Amikacin, the compromise – application is still good, but Capreomycin is still good, but Capreomycin is somewhat less, but Protionamide again is very poor.

Again, these second-line drugs, they came from Armand Van Deun, it is very recent – last year study. And also here, out of the five supranational reference laboratories, sensitivity to detect Kanamycin resistance, 100-percent at the four laboratories. Amikacin, three laboratory, Capreomycin

three, and Ofloxacin four. So, only one laboratory showed a suboptimal performance. Especially also saying [misspelled?].

And then I would like to touch at the criteria of resistance in drug sensitivity testing. So, which means that most of the clinical drug resistance actually is - largely depends on how well you calibrated to determine criteria of resistance in your test systems. So this, for example, if a sample of the clinical susceptible strains, this is a - XX indicate the drug concentration, so it will increase this direction. So you test it.

So if the clinical susceptible strain populates like this, normal distribution, and clinical resistant - clinically resistant strains distributed like this, so you can take breakpoint here. So you can - your test is 100-percent sure actually. So this is another expression of this figure, there's this cumulative, the percentage of the MIC - I mean, I'm sorry, the cumulative percentage active from concentrations, percentage of the susceptibility at the different concentration.

So, in this case this would be wonderful test, but unfortunately, there is no such test in the world at this moment. So, always an overlap with these two populations, like this. So we're supposed to take a breakpoint, either like this or we may sacrifice a little bit of the weather resistance, then we can select [inaudible] with less like this. But over - because to prevent, to use of the possibly susceptible drug for

the patient, nobody recommend little bit a low concentration. But if you think of the past reproducibility, if we take a [inaudible] with higher concentration, that would be maybe better.

Anyway, we have done in many years ago, in 1970s in Korea, so we found this Isoniazid, 0.2 mcg/ml can support these two populations most, so that's why we use 0.2 mcg/ml for drug sensitivity testing. So, here – but if you see the cumulative percentage of the probably resistant strain is increased with increase of concentration. So which means that there are many different levels of resistance.

So in this case, this Rifampicin has somewhere as flat, which means once resistant – many of them actually show high level resistance. Anyway, when we take a 14 mcg/ml, so we can [inaudible] beautiful separate this probably susceptible and clinically resistant isolate.

But if you look at this Ethambutol, it's terrible actually. This is a probably resistant isolate, this is probably susceptible isolate. So, in our system, still 2 mcg/ml can separate these two population most, but almost 50-percent of the probably clinically resistant isolate still can be classified as a susceptible at 2 mcg/ml. So how we can explain it?

And then the Kanamycin susceptibility test we have done some years ago. This is a [inaudible] spotted study. This Kanamycin in L-J medium, 30 mcg/ml, can separate the probably

susceptible and probably resistant best. But here again, we selected, with higher concentration to improve the test – I mean the test proficiency.

And then this L-J [inaudible] is here, 5 mcg/ml. But in broth, 3 mcg/ml. This we have studied in 7H – the packed egg nashot in rigid medium.

This Ofloxacin, again, as you see here, we selected 3 mcg/ml as a criteria because this concentration can separate these two populations most, but here again, almost 40-percent of the probably resistant isolate will be classified a susceptible. In 7H10 medium, even it's more. This is a broth.

So, in summary, L-J medium, as you see here in case of Capreomycin, 38-percent of the probably resistant isolate classified as a susceptible when we used 40 mcg/ml. So, Cycloserine, forget about. It's not reliable at all. Ethionamide is 27-percent, Kanamycin, 32-percent. Ofloxacin 53-percent at 3 mcg/ml. PAS, 50-percent at 1 mcg/ml. So it is very similar in 7H10 agar medium. So this is one explanation why, say like Ethambutol, over 50-percent of these probably susceptible strains still will be classified as susceptible. So, because this has been the approximate – the ratio of [inaudible] level to MIC of the wide susceptible strain of TB.

So this is one drug scale, so [inaudible] MIC and peak blood level is same. So it's very close – all those drugs, it's very close to 1, which means some of the patients probably, even if take their normal dose, probably the level of

those drug in the legion might be already self-inhibitor concentration. So how they can be selected out, which is it? So this is one explanation.

So, this is summary. So technical reliability and the clinical relevance of the Rifampicin/Isoniazid susceptibility test are very high and robust when compared with other TB drugs. But technical reliability of the Kanamycin and Ofloxacin are good actually, but there are a considerable number of probably resistant isolate remains susceptible at their critical concentration. So what you have to be careful is you have to be very careful to use those susceptible test results if a patient has the treatment history of the corresponding drug.

And then, the attainable peak blood level of many TB drugs, except Isoniazid, Rifampicin, are close to the MIC of the probably susceptible strain, making so fragile to a very small technical variation that their test reliability is poor in general. And then also the careful selection and standardization of test system and methods are essential to improve the reliability, and strict and efficient internal and external quality assurance are also essential to sustain acceptable level of the technical reliability.

So, molecular techniques should be explored to replace, or compliment the poor reliability test DST through the rigorous research on the drug resistant related genes and their

mutation site, and development of easy, cheap, and robust test systems to detect those mutations.

But we now the – we are – shifting from the conventional technique to the molecular technique, but still, those molecular techniques should be studied in terms of not only the POTP [misspelled?] resistant to identification of the [inaudible] resistant, but also the clinical drug resistance. Thank you for your attention. [Applause].

JOSE CAMINERO: Thank you, Dr. Kim. We will keep 15 minutes at the end of this symposium for question and discussion. Now we'd like to move to our third speaker, Dr. Chuck Daley from the United States. Dr. Chuck Daley has been working in San Francisco for a long time, and recently moved to a National Youth Center in Denver. Now he's a professor and head of the division of mycobacterium and respiratory infection at national youth center. He would like to present the cross-resistance between anti-tuberculosis drugs.

CHUCK DALEY: Well, first I'd like to thank the organizing committee and the co-chairs for the invitation to discuss cross resistance of anti-tuberculosis drugs. And I note that the topic is controversies in MDR-TB, and I don't see this as a controversy, but I see it as an area that lacks clarity, and it lacks clarity partly from the lecture that we just heard from Dr. Kim. Over the decades where cross-resistance has been discussed and evaluated, there have been many different methodologies that have been utilized, different critical

concentrations, different auger, whether they be liquid or solid, and different cut points – excuse me.

So, I think that that's part of the reason why there's some lack of clarity. The discovery of mutations that are associated and presumably confer resistance to the various drugs that you see here, I believe with time will bring clarity to this. So what I'm going to do today is go through, make some summary statements and then look at the studies that I think support those summary statements.

So let's look at Rifampin. In general, the data would support that there's high level across the rifamycin class. There will be, as you see in a moment, Rifabutin may be a little different in that regard, and we'll look at the data that supports that. Among the fluoroquinolones, we're seeing variable cross resistance being reported. Some of the newer quinolones that we know have enhanced activity against mycobacterium in tuberculosis do seem to retain some activity, even when there's resistance to some of the less quinolones.

The amino glycosides and the polypeptides, I think this is a particularly unclear area. In general, there's cross resistance between Amikacin and Kanamycin, and also between Capreo and Viomycin, the polypeptides, but there's various cross resistance has been reported for many years, over 30 years among the various classes, and again with mutational analysis, perhaps we're starting to get some clarity there.

Among the thiamids, there's high cross level resistance between

Protionamide and Ethionamide, and there's variable cross resistance with Isoniazid, in particular INHA mutations.

And then, Thioacetazone, I'm not going to really say much about that, but going back over decades, there's been variable and relatively low cross resistance among these various drugs that you see here. And there's now some, again, mutational evidence to explain some of this, but given the fact that that drug is not utilized very much, we're not going to discuss it today.

So let's first start with the Rifamycins. Rifampin, Rifapentine, Rifabutin, if you look at the structures here, you can see that there's one that stands out a bit differently, and you might predict might behave differently in terms of its resistance, and that's Rifabutin. Most of the work looking at resistance has been with Rifampin, and mutations in this 81 based pair region of the RNA polymerase beta subunit, rpoB, appears to confer most of the resistant as noted previously.

So this is an example of a study that looks at the difference in MICs between Rifampicin and Rifabutin. This was a study by Koga that was published back in 1998, and let me lead you through this. So you see the MICs of Rifabutin on the Y axis and then the MICs of Rifampicin here. If we use a cut point of two for the MIC of Rifampin, and then 32 for highly resistant strains, that's what these dash lines are representing.

So let's kind of go through this. Now, in this study there are 163 mycobacterium tuberculosis strains. There were 93 of those that were Rifampin resistant if you use an MIC cut point of two or greater. Five-percent of those were susceptible to Rifabutin, or 17. So if we look at this cut point here to the right of highly resistant Rifampin strains, you'll see these are the numbers of isolates that fit into those MIC categories. And this is a very interesting group here, because even though they're highly resistant to Rifampin, you can see that this is the cut point, using a cut point of one for Rifabutin, they retain susceptibility to Rifabutin, again, despite high level Rifampicin resistance.

And this is a group that's in this resistant range, but kind of intermediate resistant range, and you can see the number of isolates here, and again, anything below this line is considered susceptible to Rifabutin.

So we do see strains out there that are clearly resistant, even highly resistant to Rifampicin, that retain low MICs to Rifabutin. On the other hand, there's also this group here that show resistance to most, and you can see this is a large number of those strangers. So early – back in the '90s these strains were being reported.

So this is another study being reported by Williams. They had 29 Rifampin resistant strains. They found that 23 were cross resistant between Rifampin and Rifabutin, and that involved 18 different mutations, but 20-percent of these six

strains, were susceptible to Rifabutin, and there were five mutations that were associated with this. These are the six strains here and these are the mutations, and there's 516 here code on, will show up in multiple studies – it looks to be a mutation that is associated with Rifampin resistance, but Rifabutin susceptibility. Now, there are others, the 511 and so forth, but what you see here, or anything two or greater was considered resistant to Rifampin, anything 0.5 and less susceptible to Rifabutin. And the other theme here is that Rifapentine is usually resistant if it's a Rifampin resistant isolate. You see this one strain here that had an MIC of 1, but in general, Rifapentine and Rifampin tend to track. But on the other hand, Rifabutin is sometimes susceptible.

This was a study from Turkey that looked at 41 resistant strains of mycobacterium and Tuberculosis. Drug susceptibility testing in this particular study was using the proportion method with 7H10 media, and you can see the critical concentrations that were utilized. They found 35 isolates that had Rifampin MICs that were quite high, 32 or greater, and they were also Rifabutin resistant, very much like the first study that I showed you. But in this study, 14-percent of the isolates that had "intermediate, but resistant" MIC's for Rifampin, still retained susceptibility to Rifabutin. Again, if you use an MIC of less than or equal to 0.5.

This is that same study that's showing you – now, to the left we have the number of strains, the MICs, starting high

with Rifampicin and declining, and the corresponding MICs to Rifabutin. These are the sequences that they found, and again if you note that 516 code on position appears to show up yet again with low levels of MIC for Rifabutin and resistant levels for Rifampin. Again, there are others, the 533 has also shown up multiple times in studies. So it looks like, by doing mutational analysis, we're starting to find specific mutations that may give us a marker for when we may have Rifampin resistance, but Rifabutin susceptibility.

What about the amino glycosides and the polypeptides? I think this is partly unclear because these are multiple drugs, and they're not as similar as you might think. The polypeptides Capreo and Viomycin – you see the Capreomycin structure here, which is quite different when what we see over in the amino glycosides. And the amino glycosides themselves have subcategories. Streptomycin is a streptadine [misspelled?], which is different than Amikacin and Kanamycin, and you can see there's some difference in the structures as well.

The various mutations that have been associated with this, and there are others, also show that there's a reason for cross resistance occurring, and that both with Streptomycin and Capreomycin, just as an example, but also with Amikacin, this RRS area appears to be an area that can produce mutations to both drugs.

And if we go back to the '70s with McClatchy, prior to having mutational analysis, these associations between the different polypeptides and the amino glycosides have been described. But what you see here are Streptomycin resistant strains, Viomycin resistant strains, Capreomycin and Kanamycin, and here are the drugs and the drug concentrations in micrograms per milliliter.

So, if you look at Streptomycin strains, you see in three of the four that were tested, very high MIC to Streptomycin, but lower MICs to the polypeptides and to Kanamycin. And this is something that's been pretty consistently found over the years.

If you look at Viomycin, a polypeptide, we see high MICs both with it and its sister drug Capreomycin, but except for this one strain, lower MICs with Kanamycin, and even lower with Streptomycin. Among Kanamycin resistant strains we see a similar pattern of Capreomycin susceptibility, and again we see a strain here that has a very high MIC. And then this pattern continues. But look at this last one, Kanamycin resistant strains, the high MICs to Kanamycin as would be expected, but also to Capreomycin. This is the drug in a different class. So there's variability that's been described many times over the years.

And another study that looked at this and look at the mutations in the RRS gene, which I mentioned previously, most of the strains that were looked at here, and there were 10

clinical isolates, they could not find a mutation in that area. This 14 code on mutation has been associated with very high level resistance, and here you see a very high MIC to Kanamycin and to Amikacin, and to Streptomycin. But if we go through this and we look at the top of this table, we see lower MICs. And these are called susceptible isolates, but Amikacin and Kanamycin do not always track, as we have many times said that those two drugs are so similar that they do not have any cross resistance, but we do see that there are differences here, and among Streptomycin, the same. Now how much of this is due to methodology and poor reproducibility is unclear, and the more we pair this, I think with mutational analysis, the more clear this will become.

The final – this is a very busy slide, but I think it's very important because it makes the same point with the polypeptides. There are certain mutations, in this case at the 1401 position, in which we see MICs, high MICs to Capreomycin, but Viomycin still maintains fairly low MICs, quite a difference between these two polypeptides. These same point mutations, though associated with high level resistance among Kanamycin and Amikacin – here we see a double mutation, but we still see the 1401 and the 1401 in a triple mutation, and we see a very similar pattern when that 1401 mutation is present.

So, as we learn more and more about these mutations and we pair them with phenotypic determination of resistance, we're

starting to understand some patterns, and perhaps some predictability for the first time.

Fluoroquinolones, you all know that the Mofaxi [misspelled?] fluoroquinolones, as shown here, Moxifloxacin with this Moxiflo group added, have enhanced activity against mycobacterium tuberculosis and resistance has been associated with mutations in gyres A and B.

This is a very important study from Cannes that was published in 2006 from the Hong Kong TB Reference Laboratory. They had 132 MDR-TB strains from the time period that you see. 108 were Oflox susceptible, 24 were Oflox resistant using a breakpoint of over 2.4 mg/L, and then they put in another 11, but non-MDR strains. They use a MGIT with those concentrations that you see, and they also sequence gyrase A. So here you see 35 Ofloxacin resistant strains of MTB and on the left, the Y axis, we see the MIC of Ofloxacin, and here the MIC of Moxifloxacin.

Here you see an R value of 0.62 so there's pretty good correlation, but what's important is to look down here in this lower end of the MIC range for Moxifloxacin, and you see strains here that are above the breakpoint of the resistance for Moxi. And, so, in fact, if you actually calculate this linear regression, the MIC values of Moxi, in general, are about four times that of Ofloxacin. So if you just take Ofloxacin resistant strains, you're going to see more activity out of Moxifloxacin than you are Ofloxacin.

He sequenced these as I mentioned, and described this stepwise decline in Moxi susceptibility. So here you see gyrase A mutations, the number of strains that were looked at, and again that's 35, and so here we have an Oflox MIC of 4 that would be considered resistant, with a Moxi of 0.5, and as we go from left to right we see increasing MICs for both drugs.

In this region here, where we see Moxi susceptibility, but Ofloxacin resistance, you can see the strains here, and you can see they tend to correlate, it looks like, into a small number with specific mutations. As we move from the left to right, we now get into an area that is Moxi and increasingly Ofloxacin resistant, and now we see this set of strains correlating perhaps with these mutations. I think very importantly, this particular mutation, 12 of the highly resistant strains, resistant to both Moxi and Oflox were in that mutation, where again here we're seeing some susceptibility to Moxi with these mutations, but resistant to Ofloxacin.

I think a similar type of presentation by Cheng. This was a larger number of isolates, 138 clinical isolates, so these were the percentage of strains that you see here that have the MICs that are listed here on the left. So let me take you through this. They had 23 strains that were resistant to Ofloxacin and had no gyrase A mutations that could be identified. And then they had 32 that were resistant to Ofloxacin, but had gyrase A mutations. We see the same pattern

here. But let's look at Moxifloxacin. Again, in these 23 strains that were resistant to Ofloxacin, you can see that most of them retain susceptibility to Moxifloxacin. However, if they had gyrase A mutations, these were generally resistant. But there's a problem here, because if the cut point is used as two, you see a lot of the strains on either side of that cut point. And I can say in our laboratory at National Jewish, this is a very common phenomena for us, seeing Moxi strains with an MIC of two, and it's Levo or Ofloxacin resistant strain is that' susceptible or resistant.

So these are Ofloxacin being tested, just to give you an idea where the MICs were and what are already known to be Ofloxacin resistant, but with no gyrase. You see that the resistant MICs are here, and they shift down, much like they did with Moxi, if it's an identified gyrase A mutation.

In summary then, this looks like that non-gyrase A mutations may be associated with more susceptibility than with the gyrase A mutations, and that Moxi, you can see the shift in susceptibility when compared with the Ofloxacin.

So, finally, then, Isoniazid and the Thiamids. You can see that their structures are very similar between Isoniazid, Ethionamide, and Protionamide. We know that mutations in the INHA structural gene within INHA promoter gene have been associated with resistance in both of these classes of drugs. And it turns out, George Canetti, back in 1965 described this association. He said, "there are many wild strains of

mycobacterium tuberculosis that can contain mutants of an extremely low degree of resistance against Isoniazid. These mutants are highly resistant to Ethionamide, whereas the far more common mutant showing high degrees of Isoniazid resistance are fully susceptible to Ethionamide." So we've known about this in terms of an observation for many years.

Now, with molecular techniques, we're able to start showing why this is occurring. This was a study by Morlock published in 2003. You see the Ethionamide MICs going from very high to low, the number of isolates that were 41. If you look at a region called FA, and this is where they only found resistance in these mutations in these phenotypically resistant strains, you see they had high levels of resistance.

If you look in the INH open reading frame or promoter region, you see also high level resistance, but there are strains that kind of go all the way down to these intermediate levels of resistance, suggesting that in this area there's - if you have a mutation in this area, it confer high levels of resistance, and it's quite variable in this region.

If you look at those who had an FA or INHA mutation, again, once FA gets into this equation, it looks like it confers high level resistance, and there were some strains that had no mutations. So it looks like with Ethionamide that not only is there INH mutations that can concern resistance, but there are other areas of the genome that can also confer resistance, and it's high level resistance.

So what about this association with INH and Ethambutol? This was a small study from South Korea, 24 clinical isolates, and they had resistance phenotypically to either INH and Ethionamide or INH alone, and this was a controlled strain, small number of isolates. And so what they were trying to do was amplify a region in the 94th code on that has been reported to cause resistance in Isoniazid and Ethionamide, and then also a regularly region. What they found in those that were resistant to both Isoniazid and Ethionamide, all of them had this pattern. They could not find any mutation in this 94th code on, but they did find a mutation which was C to T, this was actually a mutation that was noted earlier in the transmission talk.

So this particular appears to be associated with resistance to Isoniazid and Ethionamide. If you look at those who are only Isoniazid resistant, there were 12 strains. They couldn't - 83-percent had no mutation in either of these two regions, but there were two that had the same pattern of mutation that was seen here, so it's not an all or none phenomena, but clearly there seems to be an association to a small number of strains between this mutation and this region in both Isoniazid and Ethionamide resistance.

So let me summarize. In terms of the Rifamycins, we usually see complete cross resistance between Rifampin and Rifapentine. There is an occasional very isolated exception to this. There's variable cross resistance between Rifampin and

Rifabutin, somewhere in the 5-20-percent of strains, and that has varied obviously from lab to lab based on the selection of strains. Among the amino glycosides and the polypeptides, we typically see cross resistance between Amikacin and Kanamycin. But it is not complete and there is more than one study out there that have shown that there can be instances where you see susceptibility to one and resistance to the other, but there are mutations that seem to confer high level resistance to both.

There's usually complete cross resistance between Capreo and Vio, but I showed you a mutation that seems to break that rule, and then there's variable cross resistance among Amikacin, Kanamycin, and the polypeptides, and I think more analysis needs to be done to literally find the pattern that helps us understand that.

Fluoroquinolones, variable cross resistance with general enhanced activity. The Mofoxi fluoroquinolones with some retaining susceptibility in the face of Ofloxacin resistance, and among Isoniazid and the Thiamids, we see complete cross resistance between Ethionamide and Protionamide, but the cross resistance between INH and the Thiamids is usually related to mutations within the INHA region, and that has been quite variable.

So, I think that what we're left with is, yes there's cross resistance. I don't think it's controversial. I think it still remains not as clear as it can be, but I'm starting to

see patterns of clarity emerge, and as we have more studies that bind the knowledge from a mutational analysis with phenotypic resistance, hopefully this will become more clear and help the clinician pick drugs. Thank you. [Applause].

MARCOS BURGOS: Thank you, Chuck. Now we move to next speaker, Dr. Pepe Caminero [misspelled?] from Spain. Dr. Caminero now is the head of the MDR-TB unit of the Union. He would like to present predictors of good and poor outcome in the treatment of MDR-TB. Doctor?

JOSE CAMINERO: Thank you very much. As all you know and all you have seen, we are learning a lot year by year regarding this topic of the management in the diagnosis of treatment of the MDR. This is because, as I told you before, this is a very new epidemic and we have very little evidence regarding of all these topics. And this is because, until now, we have not known any clinical trials comparing different regimen and drugs, and until now we have only the opinion of the different society recommendations, and most of them have changed in the last five or 10 years.

Even in my opinion – my opinion three or four years ago was very different to my opinion now, and it is because we are taking more and more evidence year by year, and for the duration, I think we must evaluate everyday what is our knowledge regarding the different topic.

For the duration, when we analyze the different outcomes regarding the most important serious [inaudible]

regarding the treatment of the end year, we can meet very different outcomes. For example, in this very good, publication, regarding the individualized management, we can have very low recollection in this very [inaudible], they are working very, very well, or in the same way, we can have this very important success outcome. [Inaudible] you are going to analyze the standardized management, also saying we can have very low rate of, duration rate, of in the same way, very high rate. Why? Because all of these patient are very different. The inclusion in all of these studies is only the patient with MDR, some cases with a lot of other resistance to other drugs or not.

For the duration, there are very, very little studies analyzing what is the best predictor of good or poor outcomes. I calculated in the literature, all the articles with this key word MDR-TB and outcome analysis. I have found 59 article, but only in 32 they are [inaudible] analysis analyzing the different factor contributing to the possibility of the outcome. And also I have made in another way, 17 other articles addressing the surgery of the MDR.

When we analyze, above all, these 32 articles, I admit 23 predictor of poor outcome in the treatment of the MDR-TB. The most important are these seven. Maybe the most of them – the more in vitro resistance to first and second line drugs. This is [inaudible] a study. [Inaudible], three year studies, [inaudible] and disease, two studies. Treatment with less than

two active drugs, one study, or use of [inaudible] drugs for three months or more, this is another study. XDR – and XDR base should knock MDR, a low body mass index.

Only these seven factors have been found in more than one article. For me, this is maybe the most important, but also I admit, this other factor meet or found in other 17 article. This is in Capreomycin resistance between the XDR, Kanamycin resistance, more drugs received regularly, having previously received treatment for MDR, male, age, low [inaudible] or clinical condition [inaudible].

Situation linkage to the patient condition, and all sorts of infection MDR-TB status knowledge at the time of diagnosis, and [inaudible] and their link with morbidity, [inaudible] initial regimen was changing due to receiving category one versus category two, alcohol consumption during the treatment, poor adherence, and [inaudible]. A lot, a lot.

Every study has found a different possibility. On the other hand, what are the 17 predictor of good outcome that I have found in this 59 article? The most important are these three: fluoroquinolone therapy with linkage to good outcome, met in three article. Absence of previous treatment with Ofloxacin is more or less the same in one article. Surgical resection, only in three article of the 17 article addressing this topic, inclusion of Pyrazinamide and Ethambutol in the regimen when susceptible was confirmed, MDR-TB cases susceptible to at least one first line drug, to receipt

appropriate therapy for more than two consecutive weeks, and negative sputum culture after two or three months.

I would like to highlight this because most of them are linked to practically two conditions, to attain or to achieve good outcome. This is surgery or the appropriate use of the anti-tuberculosis drugs. The other 10 has been patient admission, resistant pattern, younger age, hospitalized in a specialized center, older patients, new MDR-TB cases, treatment for more than 12 months, higher levels of albumin, body mass, and use of more than four drugs.

For the duration, I think we have a lot of predictor of bad outcome. We have a lot of predictor of good outcome, but if we can reduce the analysis, maybe we have the most important because a lot of them is linked to the condition of the patient, and we cannot improve the condition of the patient when we have the patient in our consultation. The most important are two, surgery and the appropriate use of the anti-tuberculosis drugs.

I would like to address the first one, what is the role of the surgery in the MDR-TB treatment? I think, at least me, I thought a lot regarding this article by the group of – where it is at the moment, Chuck Daley, for 205 patients they go to the surgery in the – 63-percent. This is a lot, a lot of them. Please, look here, the mortality attributed to surgery was 8-percent. This is very important – a very good article

identified two conditions linked to the possible good outcome, surgical resection and fluoroquinolone therapy. Okay.

When we see this article, of course the surgery was linked to a better outcome. But don't forget, a lot of mortality in this is very good article have one in the same issue of this American Journal, [Inaudible], there is an editorial by Griffith criticizing this article not by [inaudible], but speaking about okay, this is in United States with a very good surgeon with very good experience. But this represent in other cities in other scenarios?

The second one is this article of Turkey. Okay. Here is the same with surgery, the survival is a little better. Okay. But don't forget, always in all of these articles it's the same. They are sending – they are sending to the surgery, patients with these three conditions, and two of the conditions are continually localized [inaudible] disease, and if they have a relatively reverse cardiopulmonary function. Of course, if the patient has localized disease, maybe the situation at the end of the treatment could better. It could be some factor then could influence in the result of these article.

And the third one is this very, very new article published very recently, only one or two months ago, linking the surgical resection also with a favorable outcome. Only these three article are showing this comparing patients with surgery, and they had found this little improve.

Okay, but in the other way, all of these articles have been performed in places and in scenarios where most of the patients had a lot of resistance to other drugs, not only MDR, resistant to a median of six, seven, or eight drugs. In the other setting, we have this article of our friend Marcos Burgos, this is in San Francisco, and practically it's not necessary to send to surgery any patient, only to the complications.

Also, our experience in Spain in this collaborative group is the same. We never send to the surgery any patient. But because the patient we have in our hand in Spain is patients with only resistance to MDR, but one or two more resistant to the drugs, not to seven or to eight. It goes on the situation of the patient.

In this very good review, also very recent, is all the articles published regarding this topic. For me, it is very important, the last one, this. This article published regarding desparate need of the surgery in the MDR in Peru is only published in low or middle-income countries. All of them have been published in countries with a lot of resources and very good surgeons. Okay.

This is the only – for me, this is the only that we can feel to extrapolate to other settings, to other scenarios, where we have the most important burden of the MDR. This is the low income, middle-income countries, and here analyzing the

surgery in 121 cases. This is in five years. In five years, maybe Peru had more than 5,000 MDR-TB cases.

Then, they are sending to the surgery some patients, but 5-percent, 6-percent, okay, maybe it could be the ideal. And even in this situation, the situation of the [inaudible] is good, but not as good. And please, [inaudible], this is the complication, the mortality and the morbidity that this is very important.

For the duration, all the recommendation in the surgery [inaudible] this is rare in many scenarios. [Inaudible] also this is rare in other scenarios, and there is sufficient [inaudible] reserve, also very rare. But even in this situation, it must be remembered, high morbidity, mortality, and [inaudible] are not totally [inaudible].

For the duration, I think the surgery could be a support, but only in very special circumstance, like in Peru publication. But I think maybe the most important regarding the possibility to achieve a good outcome is the adequate management of the anti-tuberculosis drugs. You know, at the moment we have more than 14 drugs, and we have classified in this five group, group one, they are the best, the first line drugs oral, Isoniazid, Rifampin, Ethambutol, and Pyrazinamide, we must use all the possible here. All the possible here usually is nothing.

Second one, fluoroquinolones, third one injectable, other second-line drugs – you know, more or less. All of this

only one – in the group three only one, and in the rest until for now, this is the rules effective until now.

But okay, this is very important, but my question – this is clinical, the only clinical evidence that we have regarding the Chuck Daley talk, previously, regarding the first line drugs, what is the role of the first line drugs in the treatment of the MDR? What's its role? Can we add some first line drugs systematically to the MDR-TB treatment even if we have a very important [inaudible], yes or not?

In this article of Carole Mitnick, published five years ago, this was very important in Peru. "Inclusion of Pyrazinamide and Ethambutol in the regimen when susceptibility was confirmed, was associated with a favorable outcome."

And this other very recent [inaudible] also is the same. The patient with MDR, with the possibility to use Ethambutol or Pyrazinamide, half had better outcome than the other. Then, maybe we can have a little brawl. We don't know what is the role, but maybe you can have – this is a topic to analyze in the future. Maybe we can add many times Ethambutol, even if we suspect, but of course, Ethambutol, in the formal drugs, [inaudible].

Second one, fluoroquinolone, having analyzed it very well by Chuck Daley in the talk previously, but it is my concern, regarding the quinolone, have all the quinolones the same efficacy? What is the best? Because you – the moment – we have enough money for the Global Fund in many countries to

use the best one. And the second one, is there a total cross resistance between all the quinolones? Thinking in pharmacokinetics and pharmacodynamics, characteristics of these fluoroquinolone, we have maybe, and comparing to Isoniazid, you can see, maybe the two worst are Levofloxacin and Gatifloxacin.

Not as good Moxifloxacin even – you know, a lot of us are using Moxifloxacin. Maybe [inaudible] Levofloxacin and Gatifloxacin, but there have been only one study published until now comparing the efficacy between Ofloxacin and Levofloxacin, and this totally – the study was very concluding, Levofloxacin is better – little better, but is better. And even is a good option, Levofloxacin, in the patient with Ofloxacin resistance. Okay, this is one clinical evidence – the only clinical evidence.

I'm thinking, in the possibility of the cross resistant, Chuck Daley have analyzed very well these slides, and we have at the moment a little conclusion, maybe not definitive conclusion. In 2005, [inaudible] published one paper, one journal review, regarding the paper of the role of the fluoroquinolone process in treating tuberculosis, susceptible, and MDR.

In this 2005, maybe the most important conclusion use we cannot recommend ciprofloxacin because it's a quick drug, weaker drug. And in the 2008 publication – this is four months ago, regarding once again fluoroquinolones to treating tuberculosis, also other Cochrane review, practically always be

the same. What is the conclusion after reviewing all of the evidence that we have until now regarding fluoroquinolone? In my opinion there are four important conclusions.

Based in the possible better clinical efficacy – we have only one study, limited study, and in pharmacodynamic and pharmacokinetic consideration, perhaps the best choice to recommend for MDR-TB treatment is Levofloxacin. But however, at the different, it's not very significant, and taking into consideration the wide experience with Ofloxacin and it's cheaper price – this is very important in many, many scenarios, and better availability in the world, Ofloxacin should be considered as a good alternative, not Moxifloxacin.

And what is happening regarding Moxifloxacin? Okay, maybe it's a good drug, but we have not any evidence until now. No pharmacokinetic, no pharmacodynamic, and also, we cannot clinically study showing a better efficacy in Moxifloxacin against Levofloxacin.

The only probably justification to include Moxifloxacin in the whole essential list of medicines, was its possible use in the case with probed Ofloxacin resistance, as Chuck Daley show us previously. Also, there are no study analyzing Levofloxacin in this way, in the molecular similarity between Ofloxacin and Levofloxacin, could condition a higher cross resistance. This is only a hypothesis. Then at that moment, once we have enough money, the best option is high levels of Levofloxacin, 750 mg. And okay, if we have not Levofloxacin,

we can go to Ofloxacin, but we have not enough evidence to change to Moxifloxacin. Moxifloxacin may be only when we have Ofloxacin resistance regularly, but once again, 50-percent regarding the studies show it – but Chuck Daley – 50-percent of the Ofloxacin resistance could be Moxifloxacin susceptible. As we have the full security, we can add Moxifloxacin, but not account between the [inaudible].

What about regarding the injectable? Regarding the injectable, I have two important concern. Most important is this, assuming a similar efficacy and findings on the cross resistance, what is the best rational use of the injectables in the treatment of MDR-TB? What is better to use first? Kanamycin, Amikacin, or Capreomycin? We have a lot of studies, not too much studies, and we have some contradictory studies, as Chuck Daley analyzed previously.

The study findings of cross resistance between amino glycoside and the polypeptides are contradictory sometimes. The genotype associated with the resistance to the amino glycosides and the cyclic polypeptides are overlapping. Although emerging evidence shows a clear association between drug resistance and a specific molecular mutation, this association does not actually prove a role for the individual mutation in drug resistance. Generalizing resistance to a class of second-line drugs based solely on resistance to a single drug in the class may therefore, be misleading.

This is for me very interesting article, maybe one of the best. This is published by Ksukamura, near 40 years ago, and in the published by Ksukamura, the conclusion was, "low concentration of Kanamycin were susceptible to both Capreomycin and Viomycin. Tubercle bacilli resistant to high concentration of Kanamycin were usually resistant to Capreomycin but susceptible to Viomycin. Tubercle bacilli resistant to Capreomycin were resistant to Viomycin, but usually susceptible to Kanamycin."

Other two very important publication by Ksukamara. And the publication analyzed previously by Chuck Daley regarding the possible cross resistance linkage to the mutation in the legion mutation conditioning this resistance. I think maybe this is the best – this is the best conclusion regarding the possible use, or the best approach to use the injectable.

Isolates that acquire resistance to Streptomycin are usually susceptible to Kanamycin, Amikacin, and Capreomycin. This is very good because in more than 50-percent of the Isoniazid resistant patients, of course in the MDR-TB patients, in more than 50-percent they have initial resistance to Streptomycin, even if they have no resistance regularly [inaudible] – more than 50-percent. The [inaudible] is never an option when we are thinking in the treatment of MDR. However, rare strains with apparent single-step mutations that confer resistance to both Streptomycin and Kanamycin, have been observed, although the molecular mechanism is not known.

Maybe this is important, the most important analyzed in this way, isolates that acquire resistance to Capreomycin are usually susceptible to Kanamycin and Amikacin. A small proportion might be resistant to Kanamycin and an even smaller proportion may be resistant to Amikacin. The molecular basis for this observation have been described as Chuck Daley show us previously.

Isolates that acquire resistance to Amikacin essentially always have associated resistance to Kanamycin and Capreomycin. The molecular basis also has been described. But isolates that acquire resistance to Kanamycin show different levels of cross resistance with Amikacin. This is not the same in the two ways.

For the duration, in my opinion, the logical sequence of course [inaudible], but we never we must think in [inaudible] way. We are designing one MDR-TB treatment, never. Because more than 50-percent are resistant to Streptomycin, even if the patient has never taken [inaudible].

Then the next one is Capreomycin. Thinking in this cross resistance, later Kanamycin, and the last one Amikacin, is not the same because [inaudible] using [inaudible] Amikacin, maybe you can condition cross resistance to the other two? And in the same way here, not here. The problem with Capreomycin is the price and the availability in the market. It's very difficult. But if we have it, a very wide availability of Capreomycin maybe is the best option.

The last two question is – I have not enough time to analyze the rest of the second-line drugs, but based in the efficacy of rate of adverse events and in the price, the logical sequence in this order of second-line drugs is first Ethionamide or Protionamide, second one Cycloserine, and the last one, PAS.

And in the last group, I think we [inaudible] with this, poor drugs don't fit in this, only you have not more second-line drugs, but please, in my opinion, it is necessary to reevaluate the role of some drugs, Clofazimine, high doses of Isoniazid, and low doses of Linezolid, producing less side effects.

I think it is very important at that moment to evaluate the role of Clofazimine. In my personal opinion, Clofazimine is better drug than PAS, for example, but this is my personal opinion.

Second one, why not high doses of Isoniazid? Systematically, without account between the four, systematically, we have this very recent randomized clinical trial showing that we are having in the MDR with the same pattern of resistance, high doses with Isoniazid, we can obtain a better account.

This is my proposal, and remember the good explanation of Chuck Daley that could be cross resistance, but not always. Why not are there systematically high doses of Isoniazid and Ethionamide and Protionamide? With these two drugs, high doses

of Ethionamide and Protionamide, you would have at least one good drug, because you have some cross resistance between Ethionamide and Isoniazid, Isoniazid have a good action, on the contrary. When we are using only Ethionamide, you can have the possibility that between 15 to 20-percent of these patients with Isoniazid resistance could be also Ethionamide resistance. In this case, we can avoid.

The conclusion to this direct analysis [inaudible] not this controversial topic, this less clear topic it's okay. This is, in my opinion, the best predictor of a good outcome in the treatment of MDR is the rationale and adequate use of the anti-TB drugs available with an aggressive management of the adverse events. But we need no more regarding the different drugs. It is not the same [inaudible]. In many, many scenarios we have used a lot, [inaudible], and maybe the other drugs that I have been analyzing.

And the last one, surgery could play a secondary role in very extensively drug resistant patients. This is also my personal opinion, you go to MDR-TB project, and they are sending to the surgery less than 10-percent, could be okay, god, super. But if you go to one MDR-TB project, general project, not one reference center – the reference center is different, but you go to our country – either countries going ascending to the surgery more than 10-percent – maybe this MDR-TB project is not functioning well. Thank you very much.

JOSE CAMINERO: And we are going to the last topic. We would like to invite Chen-Yuan Chiang. Chen-Yuan Chiang comes from Taipei, Taiwan. He's the current director of the department of lung health of the Union.

CHEN-YUAN CHIANG: Thank you, Pepe. Welcome to the symposium. I would like to discuss with you the outcome analysis of MDR-TB treatment. I think that there has been improvement in the past considering the outcome, but what I would like to propose is something for your consideration. As Pepe mentioned that MDR-TB is a group of patients, not homogeneous, they have different experience in the treatment of tuberculosis.

Some of them have never been treated with tuberculosis drug, some have been exposed to firsthand and some have been treated with second-hand drugs. So when you put this patient together to analyze the outcome of treatment, you can see the reported duration of the treatment differs considerably, and also the outcome of MDR-TB vary widely. It could range from 97-percent in San Francisco to less than 40-percent in France. On top of that, the method used in analyzing the outcome in MDR-TB were not standardized until recently.

And so you see this classical paper on MDR-TB published from National Jewish Center in the United States, show us that the outcome of 171 patients, they analyzed 143 patients that 87 have response with sputum conversion. Unfortunately, some patients subsequently relapsed, so overall reponse rate was 56-

percent. But in the analysis they did not include all the 171 patients, so 37 patients were excluded, because two patients died and 22 patients they had lost to follow up, so that's the way they analyze and present the data.

There are two publications, one from Netherlands published in 2000, show us that treatment duration last for a mean of 600 days, and you see that the longest, 1,600 days, that would be 4.5 years. It's very long treatment. And publication for San Francisco with two coauthors sitting here, they show us that in San Francisco, the time that is needed for [inaudible] for conversion as a median is 14.6 weeks. But we see that the last one – some patients, they convert less [inaudible] after 3.4 years of treatment. So the duration treatment is quite long, it's quite difficult, but somehow we do not know that sort of detail, that what kind of management has been used and whether [inaudible] arrangement all the time.

And this publication, in Union Journal in 2005, proposed, speaking the same language in outcome analysis. The definition of this proposal is that we analyze the outcome of MDR-TB according to country protocol. And then you analyze the outcome at the end of the story. It means that a patient begins with treatment, and then you do not present what happened in between, whether they monitor the regimen or at what point in time sputum converted to negative, or if there was a relapse again whether did you do surgery or not – those details are not presented. So, those patients who had

consistently culture negative for the final 12 months of treatment would be classified as success or cure, and the definition of fail is that at end of the story, if you look back and you find that the patient remained sputum positive, then you classify those patients as treatment failure.

Now, the issue is, treatment failure is not defined by sputum positive at seven months, like when we [inaudible] an outcome of new patients, we say smear positive at five months, we have treatment failure, and you would register this patient as a retreatment patient, and you start that retreatment regimen again.

So the current definition of patients classified as fail, differ situations in different settings. So in one setting, patient may be declared fail if the sputum remained positive after 12 months of medical treatment. But in other settings, maybe modification of treatment will be tried and surgery may be performed, and then the treatment is extended four years, until all efforts and then you fail and give up. And the current definition, as I mentioned, does not take modification of regimen due to positive sputum into account.

Individual guidelines, it was recommended to pay attention to the response of patients. They say that patients who do not show signs of improvement after four months of treatment, they're at risk of treatment failure, and the treatment regimen should be reviewed in relation to medical history context, and all accessibility testing reports. If a

regimen is deemed inadequate, a new regimen should be designed. I agree, as a clinician, that we need to pay attention to the clinical response and if the regimen is good and the patient's response is not encouraging that the arrangement probably is inadequate and you need to modify.

But, when we analyze an outcome from the public health point of view, we also need to know how to compare patients from one setting to the other. So I would like to show you three scenarios.

Case one is a patient treated with standardized MDR-TB regimen, sputum smear culture conversion at two months, continued treatment, and was cured. And the total treatment duration, 20 months. And I think everybody probably would agree that yes, this is definition of cure.

Case two, treated with individualized MDR-TB regimen, smear converted to negative at six months, but at 12 months the culture remained positive, so the clinician decided to modify the regimen by adding PAS and Clofazimine, and replacing ciprofloxacin with Moxifloxacin, and then cultural conversion at three months, after medication of regimen luckily, and treatment continued for 18 months. After culture conversion the patient was cured and the total duration of treatment, 33 months. If the modification of a regimen was not done, probably the patient would be fail.

And let me to go to case three. A patient treated with individualized regimen, cultural conversion at six months, but

become positive again at 14 months, and clinician decided to modify the regimen by reinforcing the regimen with PAS, Clofazimine and Pyrazinamide. Unfortunately, the patient remained sputum positive at 20 months, and they decided to go for future. And sputum conversion was achieved at the surgery. Treatment continued for 18 month after the sputum conversion and the patient was cured. The total duration of treatment, 38 months. If the modification of regimen was not happen, and surgery not done, the patient probably would fail.

So, as I mention, if the treatment regimen was not modified and resection surgery not performed, outcome of patient case two and three would be fail. So it seems to me it's reasonable to classify case two and three as fail, when modification of regimen is made.

These publications from Latvia show us that the patient – different group of patients that had never been treated for tuberculosis or previously treated with second-hand drugs or previously using second-hand drugs. And you see that the treatment again lasts a median 18 months, but some of them received 37.5 months of treatment.

And these publications show us the sputum conversion increase over time, even up to 17 and 18 months, and it differ substantially between these three group of patients. These have been treated with MDR-TB drugs, this is treated with [inaudible] drugs, and this group of patients never been

treated. And you see the sputum conversion happen at different points in time during the treatment course.

The recent publication on the outcome of XDR-TB from Peru show us that the outcome of XDR-TB could be as good as MDR-TB. In their strategy, they say that patients without sputum culture – without sputum conversion after four months of treatment, drug sensibility testing was performed with the reinforcement of a regimen, defined as addition or substitution of two agents that are likely to be effective if possible, and this practice was repeated as necessary. So the outcome of XDR-TB is not different from MDR-TB in statistic analysis.

Then you go into the detail of the treatment. You see that the duration of treatment in the cohort range is 29 months. There are some patients who underwent resection surgery, you see that for the patents, the number of months from treatment initiation to surgery, the median is 11, but the interquartile range is 24, and then you may wonder, patients who have these surgeries after the last quarter range. And here is the treatment. You see that's a long duration of treatment.

So, and we know from publications that if we wait, at the end of the story, either the patient is cured, or they die, or they remain positive, or they run away. So the outcomes would continuously – the proportion of patients cured would continuously increase.

A few years ago when I analyzed the patients who received surgeries in Taipei, I wondered how should I analyze these patients? I decided to classify patients into three groups. Group A are patients who have medical – defies medical treatment failure. Those patients who have persistent sputum culture positive for tuberculosis despite treatment with adequate second-hand drugs for at least 12 months. And another group of patients that we did the surgery earlier in time to prevent their treatment failure.

And finally, it's a group of patients, that although they have sputum conversion, we worry that they might relapse in the future. So we see that the preoperative treatments of group A that we define as treatment failure, the mean is 20.6 months. So all of them have received medical treatments for more than 12 months, but [inaudible] response is nice, so it seems to me it's a reasonable intervention when the patient do not have good response to initial regimen and probably surgery could improve the possibility of cure among these patients.

And then the second group of patients that we did the surgery earlier in time and to prevent the treatment failure responses, [inaudible] selective population as like Pepe mentioned that it probably cannot, cannot do surgery for all the patients.

And in another publication that we analyzed the outcome of patients received treatment from 1992 to 1996, to have a [inaudible] outcome, we would like to know the long-term

outcome. And the way that we define treatment failure are those who remain positive or become positive again after 12 months after commencing the course of treatment, they have failed. And the patients who remain culturally negative at 18 months, they are cured. And then from that point in time we follow up, up to six years to see the relapse rate. And the relapse rate was quite low among those who have been treated with fluoroquinolone.

So, in the outcome analysis, if I allow modification of regimen, surgery, the successful rate among patients receive second-hand drugs with fluoroquinolone would increase from 59-percent, up to 70-percent or more.

So, I would argue for cohort analysis. That is, regimen failure is different from patient failure. So if modification of MDR-TB regimen is met in the consideration of potential failure, outcome probably should be fail, and the patient reregistered as MDR-TB, previously treated with secondhand drugs. This is exactly the principle that we use to analyze the outcome of new patients and retreatment patients.

So, what I would like to propose is that we consider – we classify fail, failure of MDR-TB as sputum positive at [inaudible] months after treatment, either six or four or eight, probably would depend on the regimen that you've used. If you've not included fluoroquinolone, probably it will be longer like 12 months. If you used Moxafloxacin or Ofloxacin, maybe four months – maybe six months or eight months. But if

you've used Gatifloxacin, Moxifloxacin, probably you'll need to take action earlier at four months. So that's the question that remains to be investigated.

And then the second question is whether sputum positive is smear or culture. What about patient remain - is smear negative or culture - remain positive or is smear positive, but culture negative? That kind of issue needs to be considered if we go for that direction.

And I would like to use three articles to show you the situation of sputum conversion. This is an article from Peru published [inaudible], you see that among patients - those who were cured, this shows you that - oh no, no, I'm sorry. This is the smear positive, among those who are cured is here. So you see that sputum conversion, even up to 12 months where patient was finally declared cure, this remains sputum smear positive at 12 months.

Those who were classified as cure, they are sputum culture positive at 12 months also. And these group of people, patients that received a regimen including Kanamycin and Ciprofloxacin, and we know that in Peru, Kanamycin, and Ciprofloxacin could be available in the market so that you may compromise the efficacy of the regimen.

And the study that I mentioned that a cohort of patient in Taipei for six years follow up, we see that among patients who have 90-percent of the patient we analyzed has complete information to evaluate entire bacteriological cause. 82-

percent of patients that have sputum conversion within three months, 90-percent within six months, but this entire cohort including patients who have not been treated with fluoroquinolone. And among the patients who fail, 90-percent of them remained persistently positive, and that's the reason why you use 12 months as a cutoff.

And this is a publication from Bangladesh. This regimen that they use, and you see that at three months, culture negative achieved in 88-percent of those patient.

Before we move to that direction, probably it would be useful to have a subcategory in analyzing the treatment outcome, to insure that we can compare outcome or MDR-TB from one setting to the other. So maybe we can have a set category like cure or fail with modification of regimen, cure or fail with surgery, or with both.

So in conclusion, I think current definition of fail of MDR-TB need to be considered, especially when I went to one country and they told me that they're going to use six months intensive phase, 18 months continuation phase. But when I asked them what happens if patient remain cultural positive at 12 months, they did not think about that issue. So the current definition is not useful in guiding the medical treatment or management of the patient during their treatment course. If countries forgot to consider that they need to modify the regimen if a patient remains sputum positive for certain months, probably the regimen, if they continue to use that

effective regimen, resistance would be amplified at the end of the treatment. So I will propose that probably you could see the fail could be defined as positive sputum at certain months, and that's subjective to investigation and discussion.

And patients who need modification of the regimen due to consideration of inadequate regimen should be classified as fail and reregistered as retreatment patients. With this, I thank you of your attention. [Applause].

JOSE CAMINERO: Now we have time for comments, question, and clarification.

KEN CASTER: Good morning. Thank you. Ken Caster [misspelled?] from CDC Atlanta. First of all, I congratulate all the speakers for an excellent set of presentations. I really feel I'm up to date having spent my morning here. Dr. Chen-Yuan, your proposal makes a lot of intuitive sense, however, there are reasons for modification often that have to do with delays in accessing drug susceptibility test results. Sometimes, what we find is that many settings where your treating persons with drug resistant tuberculosis, you're always starting with some guess of what the isolate is susceptible to, and then you find out what it's truly susceptible to, and that causes some modification of the regimen.

So I think you need to incorporate that into your proposal, because I wouldn't consider that as people who were

being treated with an optimal regimen to which they should have been responding to, so I'm wondering if you care to comment.

CHEN-YUAN CHIANG: Yeah. Of course I fully agree with you. In fact, the strategy is to begin with empirical regimen, and then when the susceptibility result becomes available, then you modify the regime to the susceptibility testing. And usually you would expect the susceptibility testing result would be available within one or two or three months.

At that point in time, it's not yet the point to decide or determine whether you need to modify a regimen or not, but because susceptibility testing, you prefer to modify the regimen according to the susceptibility testing. So that modification, it seems to me, is reasonable, and the modification is not met due to the consideration of the regimen may fail in the future, like when we defined the culture positive at six months, no [inaudible] response modified, then that situation is difference from at two months you modify regimen according to susceptibility testing.

So I fully agree with you that at that point it needs to be taken into consideration. Yes.

ED NARDEL: Ed Nardel [misspelled?], Harvard. This is in comment on Marcos's interesting presentation on fitness. We've been exposing a large number of guinea pigs as sentinel air samplers to patients with MDR-TB in South Africa, and finding that – and they're all MDR patients, that although infection rates are very high, progression to disease is often

limited. In fact, even some animals revert their skin test back to negative after having been infected. And about half the organisms are catalyts negative. We don't know about the S315 mutations yet, but in talking to Ian Orm [misspelled?], he's been using clinical isolates and finding in the medicine chamber, that, in fact, he gets quite a bit of pathology from drug resistant strains, and I think the difference may be a dose phenomenon. I think it's about 28 organisms he said in the lungs of the guinea pigs he exposes, where we think we're getting very few in natural exposure.

So the point is that maybe there is a difference between what we do in the laboratory and what happens in actual natural exposures where the doses of these organisms are quite low.

MARCOS BURGOS: Yes. I agree. I think that one of the implications of our study in macrophages is that maybe this resistant mutants developed by capacity to grow faster in the macrophage model, but this doesn't imply that in the community maybe they're more transmissible.

SHADA FIRKSRA: Shada Firksra [misspelled?] from Turkey. I'm working with MDR so I want to share one of my experiences with them there. Some of my cases, let's say nearly 10 of them, have left the treatment for one or two or three months then with the help of the sponsors they come back, and in this short time, although they use Ofloxacin and Amikacin during treatment. They all became positive. So,

different from the first time regimen, with this regimen, if you stop the regimen, in a short time, smear and culture become positive. That's one thing I want to share.

The second thing, about the last speech, about classification and terminology of outcomes, I think there is something I should stress on that. Adding two drugs, although we all know that for that indicates, adding one drug is not feasible, but adding two drugs is not also feasible. Adding is not feasible. What we need is we need to create a regimen at a certain point of time where you have a patient and the patient failed, so the point is you should have a stable or successful regimen, not adding these drugs or other drugs.

So, adding two drugs is not acceptable also. That may be the last option, and you should do it okay, but please don't mention it as I know.

One last thing, I want to add that although we had laboratories, we have problem in getting drug susceptibility testing on time and with quality. So mainly, we all depend on the failure status of the patient, and observation of the treatment previously. If the patient really swallowed the drug for six months and failed, then we go to second-line regimen. I think that's something I saw for Lima as recently as yesterday because they had the susceptibility testing came in five months. That should be important point because I think we need molecular tests to get very short time to begin this kind of treatment.

CHEN-YUAN CHIANG: Yes. What I'm trying to present is that what is happening at a moment in different settings, like in Peru, in Latvia, and in other countries, that the strategy that they use is to modify the regimen from time to time, and at end – so, in some settings they do the modify and some settings they modify, so the result is incomparable in my opinion.

And also, some people forgot that they need to pay attention that at a certain point in time, it really is important, either you stop or you modify, because if you continue, the regimen is not possible to cure a patient. All you got is to amplify the resistance. So I am not proposing – I am not emphasizing we need to modify. What I'm saying is that if modify, modification regimen happens, if the process of the patient has failed because you lose your confidence on this regimes – those are the reasons that you modify the regimen. So, it's a regimen fail or the patient remains possible to be cured. That's the point.

JOSE CAMINERO: I fully agree with your last comment. Adding one drug, but also adding only two drugs – initial drugs of, of course, will be not the fluoroquinolone and [inaudible] also it could be a mistake for the reason many times I have told that I don't like a lot – I don't like too much, the individualized management basis in the second-line drugs [inaudible]. Why because you have the result after five or six months usually in the field, what is happening in the moment?

You can have, at the moment, sensitivity to one drug that you think may be resistant. You have seen the presentation of Sang Jae, you cannot believe in the Kanamycin and Ofloxacin sensible [inaudible] the resistant result. We have more than 30-percent of possible different results between the clinical relevance. They are not the same, the reproducibility in the laboratory as the reproducibility of these drugs – okay, they are very good, they are [inaudible] relevant.

For the duration, in many [inaudible] scenario, I am very afraid regarding to use individualized regimen regarding the VST result, because you can think to change the regimen at four or five months, what, one drug, two drugs? Okay.

RANDAL REESE: Randal Reese [misspelled?] from Denver Public Health Department. The question I have is, is there a role for the animal models in looking at some of these issues? For instance, the patient with Rifampin resistant strain that the laboratory says looks like it's Rifabutin susceptible – I think we always feel uncomfortable with that. And you'd never be able to look at that in a human clinical trial, but is there a role for the animal model in looking at some of these disparate results?

CHUCK DALEY: I think yes there is a role. These are expensive studies to do, so it's always going to be prioritizing what studies you want to do. I don't know if Jacque is out in the audience, or Eric Nuremberger

[misspelled?], people who have experience with animal models,

and whether they would agree. But it does seem like that's the way you would try to look at this to see whether or not having in vitro susceptibility to Rifabutin and resistance to Rifampin demonstrates activity in an animal model, because that's the question clinicians are always asking.

Okay, it says it's susceptible, but can I use it? You know, traditionally we've said to use it, but don't count it. That's kind of been the same thing with the fluoroquinolones. If you have Ofloxacin resistance, give Moxi or high-dose Levo, but don't count it. Eventually if you do that, you end up not counting anything, so we do need some additional evidence, I think, as clinicians. And an animal model might convince us a little bit more than what we just have now.

MARIA RODRIGUEZ: Maria Rodriguez [misspelled?] from Dominical Republic. Regarding the definition of failure, I would like to add the comment that also, following the recommendations of the 2006 guide, some countries are classifying as failure, also patients who have to be suspended on treatment because of side effects. And for me, that is not a good definition, because it's not a failure of treatment – it's not a pharmacological failure, it's a clinical failure, but not a pharmacological failure.

SANG JAE KIM: Thank you of your comment. I think that maybe probably we can bring all these comments up to WHO meeting or [inaudible] committee to –

CHEN-YUAN CHIANG: Yes. I fully agree. I have discussed this topic for some time with Maria, and it's okay. We are thinking in a failure, but in many, many times, when we are working with the patient, we are thinking in the pharmacological or bacteriological failure, not also in the clinical failure. Maybe we must think to classify a little different, [inaudible] I fully agree.

MALE SPEAKER 1: This is a response to Pepe's comments on individualized and standardized therapy. Certainly, there's no one way to treat MDR-TB, but at Partners in Health we have pretty much used individualized therapy both in Peru and in Russia and other sites. And I think where no one's adding a failing agent to failing regimen, that would be follow, but certainly adding when you get back results and you've started with an empiric regimen. And you find out some of their drugs are likely to be resistant, it seems logical to replace those drugs if you have good replacement drugs with others. And that's basically what we do, and as you know, the results even with XDR-TB have been quite credible, so I think at least there is some support for that approach I think.

JOSE CAMINERO: No. I agree. We need individualized regimen in many place, [inaudible] the patient with TB in second-line drugs in the past, but I tell you, we must consider a lot the history of drug taking in the past. It is very, very – it is key because for me there is no problem like who in your project where you have a lot of [inaudible], but lately, I have

seen in the field, many, many, many scenario where the person in charge of them, the MDR-TB patient received – resistant to Ethionamide, okay, put him off Ethionamide – do you understand? Of course, individualized, if you have the very style specialized physician, it's a very, very good approach.

[Inaudible] second-line drugs in the past, and even in other country where they have been using second-line drugs in the past widely. But the interpretation is key always, and for the duration, in many, many cities, sometimes I am afraid – I am afraid – only I am afraid.

MALE SPEAKER 1: Hymie tells me that in Peru now there's a wide use of a very strong standardized regimen with very good results, but it means you use a lot of drugs up front.

JOSE CAMINERO: Yes, of course.

MALE SPEAKER 2: Thank you. Don't you think that one of the difficulties of trying to have standardized definitions is because we don't have standardized regimens and we don't know the duration of treatment, it is individualized, etcetera? And secondly, I would have thought that one of the controversies would have been to argue for or against individualized and standardized treatments.

MALE SPEAKER 3: I would like to ask your expert opinion about small, but disturbing group of patients who seems to be perfectly well, or almost perfectly well, who also got their sputum examined on some obscure occasion due to some

obscure reason, and still grows MDR bacteria. I just recently have a patient – we took 10 sputum in her, she was clinically okay, and she was from the Former Soviet Union. She had MDR growing in one sputum. What is your opinion about this?

JOSE CAMINERO: I think it's very complicated, sometimes, these cases, but don't forget, you have a very good – an important improvement even in, and in the clinical part of the patient. Maybe the patient is going well, but it is dependent of the culture that you have. You have a culture, for example, of being of three, four, five months, with a very important number of colonies showing MDR – the patient is MDR.

And even if this patient [inaudible] – experience is very good, but this patient has very important possibility to relapse. You have a very important growth of colonies with MDR, even if the patient go well, you must change the treatment to one MDR-TB regimen. It is very difficult if you have at the end of the third or fourth month, only very few number of colonies, less than 10 and 20. This is very different, because in this cases, could be the transitory resistance, you are killing the last part of the bacilli population, and in this situation you are killing the most resistant population.

If you have only one culture, or even two culture, or a very few number of colonies, please, don't take [inaudible], please follow this patient with more number of culture. But if the rest of the culture is showing you increasing numbers of

colonies, this patient is failing, even with the clinical and [inaudible] improvement.

CHUCK DALEY: Two things, one is, I would say if they had 10 sputum, somebody was looking for something, so I mean, somebody must have been suspecting that they had TB to have 10 sputums sent. And then when you do that and you have one that's positive, then I think you still have to go back and say could it be a cross contamination? And so I would go back to the laboratory and then try to figure that out.

And then the next issue was how was a culture and how was the drug susceptibility pattern, or how were the DST done? Because if it was done only in a liquid system, we get several of these a year which are actually mixed infection of Mack [misspelled?] and drug susceptible TB, that are sent to us as MDR-TB. And then we put it in auger place, we see different colonies, separate them, and you see that one is Mack and one is drug susceptible TB.

So, I mean, those are some of the things I would think about. Cross contamination, mixed infection, and why did they have 10 sputum? Because somebody was looking for something. They must have seen something on an x-ray that would make them suspect TB.

JOSE CAMINERO: We take the last question, and then we close.

FEMALE SPEAKER 1: Thank you. China has decided for first line standard MDR treatment to use Levofloxacin, to use

Amikacin, Ethionamide, they don't have Cycloserine, PAS usually only available in drip, means it's impossible for two years – we see that many of the known MDR patients have already taken some of those drugs in an irregular way, and many of those do not respond. What is the alternative after this level Amikacin, Ethionamide, if they don't respond?

CHEN-YUAN CHIANG: In China there is – we have a very important problem in the relay community regarding the most importable problem is because Cycloserine is not permitted in the country, and it is, in my opinion, very dangerous.

We do not Cycloserine in the way you are using the other drugs, but also with very little availability of PAS also is dangerous. And when you go to the field, you can find a lot of patients have received fluoroquinolone in the past, and this patient cannot go to standardized treatment because the standardized treatment is more or less for well for the patient who didn't receive previously second-line drugs. This is a lot of mistakes – this is very important to address, because if not going, you will have Cycloserine maybe could be very late.

JOSE CAMINERO: I think probably I can conclude this symposium by one statement, and that is considering the management of the MDR-TB there remain several issues that need to be addressed in the future. With that, I would like to thank all the participants for coming to this symposium and stay to the end of this symposium. Thank you. Have a nice conference. [Applause].

[END RECORDING]