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THE BASICS OF HIV RESISTANCE TESTING

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[video transcript]

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Today's speaker Dr. Antonio E. Urbina. Dr. Urbina is the medical director for the Mount Sinai Institute for Advanced medicine downtown clinic in New York City. He also served as medical director for the clinical education initiative of the New York State Department of Health AIDS Institute as well as professor of medicine at the Icahn School of Medicine at Mount Sinai. Since completing his residency in internal medicine at St. Vincent Catholic Medical Center in Manhattan in 1995, Dr Urbina has pioneered innovative educational programming for community based clinics, hospitals and public health departments. He has directed more than 10 HIV clinical trial research protocols. From 2007 to 2009, Dr. Urbina served on the Presidential Advisory Council on HIV AIDS as well as Governor Cuomo Task Force to end the AIDS epidemic in New York State. Thanks so much for joining today, Dr. Urbina. And now I'll let you take it away.

01:05
Great. Thank you, Tara, for that very nice introduction. So today's presentation is going to be on the basics of HIV resistance. And you know, luckily, I think we're dealing less with resistance, as our therapies become more potent, and have these high genetic barriers to resistance. But, you know, we still have cases of patients that have acquired multi drug resistant virus, or through this imperfect adherence have actually developed drug resistance. So I'm just going to take you through what some of the basics are in terms of how to move forward and how to interpret resistance tests. So these are my disclosures. And the learning objectives are going to be to state the definition of resistance and discuss the causes of HIV drug resistance, discuss the test used for measuring HIV drug resistance, including, you know, genotypes, phenotypes, and then also tropism, testing or profile testing. And that's if you're going to use one of the entry inhibitors called Morel rock. And then describe the indications for when to perform resistance testing and review which tests are appropriate for optimal patient outcomes. So before I start on that, I just want you to know that the way that we develop resistance is two ways is one is that we need ongoing viral replication. So we need virus that's replicating. And then to what you need are drug levels that are either sub optimal, so maybe just not enough args or drug levels that are kind of fluctuating like this. So those two elements create the perfect storm for how to breed resistance. So for example, if someone with living with HIV stops taking their meds, viral load goes up, they've got ongoing, ongoing viral replication, but they don't have that selective drug pressure. So it's really those patients that are imperfectly non adherent, where we really get really bad mutations, and where it gets more difficult to treat the patients. And oftentimes, those are patients that we don't know. And that can be patients that maybe stop their meds during the weekend. Take them every other day or every third day, or maybe if they're on multiple air, teas just take two or one of the compounds. So that's just something for you to know that's important. And also, importantly, is if someone should stop their AR T's because they're hospitalized, you know, they can safely stop them. And then once they're kind of acute medical illness resolves, then you can safely resume back to medications, and they'll re suppress. So the recommendations for resistance testing for the DHHS guidelines, so genotypic testing is, is

recommended at baseline and we'll go over what that is. And then genotypic testing is also recommended at first and second failures. And then really anything beyond that what you want to include his phenotypic testing, make sure to include testing for integration resistance, and then possibly also profile testing. If you think that you may need an entry inhibitor to full to actually concoct a fully suppressive regimen so genotypes first and second failure. Baseline first and second, and I would say maybe, maybe at second Canna failures, you may want to consider doing a phenotype in addition to the genotype. And there is only one company that really does the phenotypes. That's the monogram company out of San Francisco. And in order to include testing for integrase, you would do a fino sens GT with intergrates. And that will do your genotype, your and your phenotype against all of the enzymes of interest. And then again, profile testing, only if you think a patient has really severely multi drug resistant virus, and you may want to consider use of an entry inhibitor. And really the the one

05:45
that's available is mihrab. Rock. All right, so mutations, what are mutations where they occur during replication. And oftentimes, the virus because it replicates so quickly makes its own mutations that are not significant or clinically significant. And those are called polymorphisms. But the significant mutations change the structure, the shape, the characteristics, and behavior of the virus. Mutations alone or in combination affect the mechanism of action of HIV drugs. Some mutations are the result of selective pressure of drugs, and mutations that result from one drug can affect other drugs. And mutations can impact the fitness of the virus. So that's why even though we have a lot of anti retrovirals, we only have several classes of AR t. So the longer you keep somebody on a failing regimen, the more likely they are to kind of develop deeper mutations that can impact the whole class. So that's why, if you first noticed detectable viremia, you know, 9.9 times out of 10, it's going to be because of a lapse in adherence. So what you want to do is counsel patient and then repeat the viral load in a month. If it's still detectable, and you kind of have some sense that the patient is being adherent, then you want to draw your resistance testing during that time. Have the patient stop their HIV medications, and then when the results come in, that's when you can then decide what's the best next step in terms of sequencing of their AR t. And then some mutations can impact fitness. So the one is that M 184 V, which I'm going to talk about very shortly, but that one actually cripples the virus and can affect its ability the virus's ability to replicate. Okay, so identifying a, a, a mutation. So here we have the M 184. B. So M is the amino acid Muthiah name. That's what's seen in the wild type virus. And 184 is the codon position in the reverse transcriptase gene, where this is found and then Bailey, is the mutant amino acids. So that one may be through poor adherence through selective pressure, this Muthiah Annie mutates and you get a veiling in its place, and that's the M 184 V mutation. And we know just by just anecdotal and historical samples, that that one confers resistance to three TC or FTC, Lambay, padeen, or emtricitabine. And oftentimes, what you'll see sometimes in a genotypic report is mixtures. So mixtures are highlighted by this kind of diagonal and slash b. So what this means is that in this patient sample that there's both wild type and mute variants that are present, and what that likely means, and it suggests is this kind of wavering adherence. So again, mutants mixtures, mixtures are typically a sign of impending doom deeper resistance, indicating that they still have some wild type virus, but this newly mutated virus is also emerging. So again, mixtures really are the first indicators of this emergence of resistance. So genome tipic testing really predicts

structural changes. So remember, HIV is an RNA virus and RNA is translated into the amino acids. But in order to do that, HIV comes equipped with reverse transcriptase that transcribes then this RNA into DNA. Then from this DNA is where we get the programming for these amino acids. And that's what a genotype shows you that it shows you this linear sequences of amino acids. But that's not really how this protein works. It's not linear, it actually folds

10:40
and forms these processing and foldings. So a genotype tells you what this linear sequence of amino acids is. But it needs an algorithm in order to predict what these amino acids sequences, if there are mutations, how they can impact sensitivity to antivirals. So a genotype basically gives you the linear sequences of amino acids. And then all of those are plugged into an algorithm. And then that algorithm is based on Match samples from the lab, to give you this prediction of whether the this patients virus is going to be sensitive to any of the antiretroviral therapies. So this is an important distinction. genotypes and their algorithms are good, the more samples that are matched, it increases its intelligence and its predictive values. That's why genotypes are very good early on at baseline or first failure. But if you have a patient like multidrug resistance, heavily treatment experienced, you know, it doesn't really give you as predictive information as a phenotype. Because the phenotype actually grows the patient's virus in a petri dish. And then looks at these kind of concentration curves, inhibition curves of all of the antiretroviral therapies. So that gives you more of an interplay of like how these mutations really interact with each other in terms of actually being able to grow or be inhibited by these antiretroviral breaths. So a couple of other terms for mutations and drug resistance. So we often speak about drugs having a high or low genetic barrier to resistance. So a low genetic barrier to resistance is when a single mutation can cause resistance to a drug. So as described before, the M one ad for V, that knocks out three TC Lambay VD or FTC emtricitabine. The K 103 N knocks out the first generation NNRTI. So drugs like a fabrics, and developing, and so on. So you'll see they're examples of drugs where just a single mutation can knock out that drug. So those drugs have a low genetic barrier to resistance. Fortunately, in the modern era of HIV, we have drugs now that have a higher genetic barrier to resistance. So drugs like boosted darunavir drugs like dou u Tegra. Vir, big tech Revere, those drugs have a higher genetic barrier to resistance, meaning that they need multiple mutations in order to confer complete resistance. And that's great, because that means that it gives us a little bit or patients a little bit of a buffer. In particular, in times where they have some lapses in adherence. You know, they've always said, Hey, patients need to be adherent 95% of the time with drugs with a higher genetic barrier to resistance. This allows for those kinds of Miss doses not to lead to significant drug resistance. You know, with that said, we still want to recommend to our patients to take your medications daily. And resistance is not an all or nothing phenomenon. It's a continuum, from full susceptibility to full resistance. And that's kind of what a phenotypic report tells you. Gives you the full change resistance. How much more resistant is this patient's virus compared to a wild type virus? And sometimes that's important, in particular, in patients that have multi-drug Resistance have been cycled through various anti retrovirals for many years, maybe have been sequential monotherapy was added, that's one of the worst things to do is just to add one drug to a failing regimen. Is that a very easy way to confer resistance. So again, whenever you switch a patient, you want to switch them ideally to at least three drugs that they're going to be fully sensitive to.

15:34
So, here we have an example of a genome typic test. This is the genome short prime from monogram biosciences, a subsidiary of LabCorp. And this is the result that you'll get back. So what it does list is the categories of anti retrovirals nucleoside, reverse transcriptase inhibitors, non nucleoside, reverse transcriptase inhibitors, integrase strand transfer inhibitors, and protease inhibitors, it gives you the generic name, the trade name, and then it lists out the linear sequences of amino acids. So here they have the M 184, V. And here we have the K 103 N, the y one, Ada L. So what it does is that it plugs these in to then its algorithm or AI. And that's where we get this prediction of if this mutation is going to confer resistance, and to which a B AR T drugs. Remember, this is not a true phenotypic report. It's not growing the patients virus and actually stressing them with the anti retrovirals. This is an algorithmic model. And again, newly infected patients baseline first failure, this is typically enough, and you can really trust these algorithmic interpretations. So talking a little bit about switching patients. You know, we do want to be careful when we switch patients to new anti retrovirals. So and those that are suppressed already, we have to do our archaeology, meaning we have to get When were you diagnosed with HIV? When you did start on antiretroviral regimens, were you always virologic be suppressed? Did you have to switch your therapy because of resistance? And if so, did you have a resistance test performed. So you definitely want all of that information before you switch patients to a new regimen. Because if you switch patients from a high genetic barrier regimen to a low genetic barrier resurrect regimen, and if they have archiv resistance, then this new regimen is not likely to lead to full virologic suppression, and that patient may develop virologic breakthrough and resistance. Remember to whenever a person develops resistance, that mutation is archived and hiding somewhere in that patient's reservoir, and it never goes away. So whenever you have documentation of resistance of a mutation, you always have to factor that in when you're choosing a new AR T combination. And this data really comes the first one is from the switch Mark study. So this was a study that looked at switching out patients that were on a boosted protease inhibitor, and in this case, it was called lopinavir to a drug called rotogravure. And now Tegra. Vir is an integrase strand transfer inhibitor. Yes, it's potent, it's tolerable, has a more favorable lipid profile, however, it has a lower genetic barrier to resistance. So in this study, what they did is that they took patients on boosted lopinavir and two nucleoside reverse transcriptase inhibitors, and then they randomized them one to one to either continue on that regimen. Or to be randomized to this lower genetic barrier drug route Tegra veer plus two nucleus science. So in order to meet the inclusion criteria for this study, patients had to be viral logically suppressed, on a twice daily regimen of a boosted lopinavir with two NRTIs and Then they randomized the patients to either continue or to switch. And their main outcome was looking at the effects on lipids. So they wanted their primary endpoints where their mean percent change in the lipids at 12 weeks. And their secondary analysis was the proportion of patients that maintain virologic suppression and they wanted to maintain a non inferiority window of that had a 12% margin. And, surprisingly to the investigators what happened. And that's why the study had to be interrupted early is that those patients that were randomized to the route Tegra Veer, so a lower genetic barrier drug had actually higher rates of virologic breakthrough or lower rates of maintaining virologic suppression.

20:55

Um, so again, and this was likely due, in fact, to archived resistance, that the patients were harboring that that were not evident during switch to the patient was able to be suppressed on this boosted protease inhibitor. However, when they got switched to a drug with a lower genetic barrier, that's when that archive mutation didn't have enough pressure of the HRT drugs, and the patients broke through. So what are some of the benefits and limitations of genotyping? Well, one, it's less expensive, faster turnaround time. So we spoke about the mixtures, but it's better at predicting resistance. If there are mixtures presents, remember that M 184, and V. So for example, for a phenotype, you have to have 40% of the viral swarms have to be the M 184. V, before that phenotype is able to detect resistance. Whereas with the genotype, plug it into an algorithm, and it's going to predict resistance to either three TC or FTC. It's also good at identification of variants that reflect prior resistance. So again, kind of the genotype are kind of like the footprints that tell you that there's a bear in the woods. So genotypes are those footprints. And that's the genotype and the phenotype is actually the bear seeing the bear in the woods. So again, genotypes can tell you a little bit more or can predict earlier resistance. So their major limitation is that that algorithm kind of breaks down a little bit, if there's very new drugs, and there's a lot of knock if and if there's not a lot of samples with these mutational patterns. Or if there's just a lot of mutations, then that algorithm kind of breaks down. A phenotype again, is the bear actually in the woods, it's a direct measure of susceptibility is like an anti bio gram. Those are for anti microbials antibiotics. But this is an antiviral gram for antivirals. It detects the impacts of some mutations that are missed by genotypes, again, so where the genotype may miss something, the phenotype can't detect it. It also demonstrates all the effects of complex combinations of these mutations and how they interact with each other. So resistance and cross resistance Resensitization partial activity and also gives you a sense of the replicated capacity of the virus. It's very easy to interpret, which is what I'm going to go over. And its major limitation is cost. So it's about $2,000, you need about 1000 copies, maybe sometimes you can get away with 500. So it's about two bucks a copy to have it performed. So genotype or phenotype or both. All right, so we spoke about this. So, genotype can identify resistance when mutations are present only as mixtures, therefore, it can give an early warning sign that resistance is on the horizon. The phenotype directly measures virus versus drug it's a it's a true measure of susceptibility. But both tests in combination may provide the most complete picture of resistance. And this approach is most useful in patients who are more treatment experience. So just to review, again, genotype less expensive, shorter turnaround time, appropriate for treatment, naive patients and early treat We've been failures. The rules used to define resistance may not be fully developed for newer agents. So as newer agents come in like the habitat groovy or the slat, Travie, or maybe Lenna Caprivi, or they may not be as reliable. And when multiple mutations are present, the interpretation may require a complex algorithm.

25:27

So, advantages disadvantages of the genotypic test. So, here's one where I want to show you and this is an example of that fino sense GT, I'm going to go over it with more detail, but here is the genotype. So it's that linear sequences of the amino acids, and here we have with a translocation, so that M 46 i In the protease gene. And then that slash represents mixtures. So here, the genotype is predicting that the drugs are that this drug Sequenza Vir is not going to be effective against this patient's virus, but the phenotype which is actually growing, the patient's

virus is saying yes, so actually, the net assessment is that the drug Sequenom, Vir can be used, not that you would use this drug but can be used. And it'd be a fully active antiretroviral agents. So again, these are called discarded rules. But if you just looked at the genotype here, and plugged it into the algorithm, it would have said no. So advantages and disadvantages of the phenotype test, again, it's a true measure of target enzyme susceptibility. It's very easy to interpret. And again, what you really have to do is just look at the net assessment. And that will tell you whether a drug is going to be effective or not, and how effective it can be. Because it does demonstrate the effects of these complex influences and what these combinations of these music of these mutations do in terms of resistance, cross resistance, Resensitization, and partial activity. So for example, the M 184 V. Although it impacts three TC and FC, and FTC, it hyper sensitises, TDF and tf. Um, so it's something that you may want to keep on board because you're going to increase the length of the TDF or TAF. Sometimes, you want that just to cripple the virus and to maintain virologic suppression. And again, I would say it's an underutilized test. But I, I would say even second failures, but definitely beyond are heavily treatment experience complex, get that phenotype, in addition to the genotype, and that's called that fino sense GT with integrates. So some of the major limitations, it's, you know, the longer turnaround time, more expensive, and sometimes difficulty with interpreting these mixtures, like I said, maybe 40% of the mixture for the M 184. VM to be that V or else that phenotype won't, won't be sensitive enough to detect that. So here's one indication of maybe where the, there is, again, a discordant. So here, this is the phenotype, and I'm going to go over what this means. But typically all blue here, this is the full change, which I'll go over. These are the genotypic mutations and linear sequences of amino acids. So we know that k 103, K N confers resistance to the first generation NRTIs, like nevirapine and fabrics, however, with this mixture, the phenotype is saying that it's sensitive. However, we know, just based on samples and laboratory testing, that any k 103 is going to confer resistance. And that's, in fact, what this net assessment is telling you. So again, sometimes we have these discordant rules, which I'm going to go over but you're you're not really going to have to remember that because this net assessment is going to give you the correct interpretation. So let's talk about the finos sense GT with integrates really. So the phenol sense GT and this is what it does is that on the front end, it takes out. We begin to patients virus and extract from the patient's virus, most of the poll and a small portion of the gag G, we then take that patient derived segment and inserted into a resistance test factor.

30:21

It's a well characterized lab strain of HIV one. The envelope of the test vector, of course, has been destructed deconstructed by luciferase, which is then going to be an indicator gene that we can use later on to measure light counts, or measure when we start to make copies. We then take that resistance test vector, which harbors the patient's HIV, DNA, and we co transfected with this Murine Leukemia virus DNA. And that's because we, we do not have an active envelope within our vector. And then the resistance test factor and this Murine Leukemia virus DNA envelope are then transfected into this human embryonic kidney cell, and they undergo single cell cycles of replication. Remember, because it's got this luciferase gene, now it's going to light up when the virus is able to make copies of itself and not light up when it's inhibited. So then antivirals then are added within the lifecycle of the virus. And then, and then we measure these counts, using these relative light units that are clicked on by this luciferase

gene. And then we make these curves. So these are the curves that are made. So these are the concentration curves, and they're all compared to a control virus. That is wild type. All right. So the IC 50, then, is the concentration of drug required to inhibit viral replication by 50%. The full change compares the IC 50 of the patient to the icy 50 of the control, and the higher the full change, the more resistant the virus. So here, the graphs are super imposable. So there is no full change. Here's an example where we have reduced susceptibility. So anytime a patient's curve is shifted to the right, a full change has occurred, indicating increasing resistance, therefore a higher concentration of drug will be needed to achieve this i i See 50. And then again, it's the patients I see 50 versus the control, that then will give you this full change value. And I'm going to demonstrate that on a genotype and just like as drugs as the curves can shift to the right. Remember I told you about that M 184 V it can hyper sensitized TDF we can see hyper susceptibility or so a shift in the patient innovation curves towards a lower drug concentration is indicative of increased susceptibility. So here, we have a patient and the graph shifts to the left meaning less drug is required to achieve that IC 50 hence leading to hyper susceptibility hypersensitivity. So hyper susceptibility, there has been independent trials that have shown hyper susceptibility not just to drugs like a Fabrice but also to no food, beer, and AZT. So that's good because we can use that regimen. That's why oftentimes patients that just fail with the M 184. V. When you sequence then you're able to keep on drugs, the combinations of like TDF FTC or Taff FTC with either a boosted PII or a second generation enter grace and maintain virologic suppression because what you're gonna have is two and a half drugs on board. And for early failure, that's often enough. So this is the fino sense, GT plus intergrates, combination phenotype, genotype test. And I'm just going to unpack this a little bit but you see that The Blue the gray, what you really just need to know is the net assessment, which I'm going to show you in the next couple of slides. But remember, this phenol sensitivity is a combination of a genotype

35:16

and a phenotype. And this report integrates all this information into both of the test. So what I want to show you here is the fino sense GT test report. So again, gives you the class of antiretroviral. So nucleoside, reverse transcriptase inhibitor, these are all the drugs available in this class gives you their generic name, and then their brand name. These are the cut offs that are predefined based on laboratory values already. So the lower clinical cut off, and the upper clinical cut off. So I am going to tell you what these are. So lower and upper clinical cut offs are several drugs, the lower cut off is when that full change at which you start to get resistance. And then that upper point is where beyond that really just the drug is not going to be effective. You'll see that some antivirals have both the lower end and upper and others just don't have it, they just have this median cut off. This then gives you that full change remember the patient's IC 50 over the wild type Ic 50. So again, that full change then, compared to what the laboratory base cut ups are, will tell you then the degree or the phenotypic degree of resistance. So for example, here, you'll see these little whiskers here bow ties. So for drugs that have a lower and upper, you'll see them here. So once the drug is full change goes beyond that lower cut off, then the graph will have these little hashtags lines through it. And then if the full chain is above the upper or that median cut off, then it goes to dark gray, or black, indicating phenotypic resistance. So for example, let's use this drug a back of year, it's lower clinical cutoff is 4.5, it's upper 6.5, this patient's full change. So their IC 50, over the wild type is 5.99, fall somewhere in the middle, so

partially sensitive. Look at to know for beer here, so we know the lower clinical cutoff is 1.4, the upper is for the full change is 2.53. Again, partially sensitive, and these others are fully resistant. So again, it gives you the lower and the upper cut offs gives you the zone of intermediate resistance. And importantly, it gives you this net assessment to really address any genome or fino discordances. Because you do see here that like, sometimes the genotype based on these mutations is saying no, it's not going to be sensitive. And this is saying that it's partially sensitive. And these are based on these discordant rules. So again, these are the cut offs, historical cut offs, these are the baselines, this is giving you that zone of intermediate resistance. This is telling you what the genotype is reading this is fine you what the phenotype is reading, but all that you really have to look at is this net assessment. So it makes it very, very, very easy to interpret these, these assets. And then again, you'll see little footnotes describing why these dis what these discordant rules rules are and how they came up with their final interpretation. You also have at the back a summary page of drugs that are sensitive, partially sensitive and resistance. So this is kind of a quick reference. And it also gives you a replicated capacity measurement here based on wild type virus, you assuming a virus that's wild type is replicating at 100%. So let's let's say someone has a real, severe multidrug resistant virus and let's say if you've run out of options that you may want to just really cripple the virus enough, get a low RC, just so you can maintain some virologic suppression, cripple the virus and hopefully not allow the patient's HIV to pass Rest. And then lastly, this last page has a wealth of information that includes a complete list of mutations, the summary of all mutations that were observed including the polymorphisms, etc. So something just kind of ticked Landsat.

40:22

Okay, so phenotypic susceptibility cut offs just to highlight that again, again, so that lower clinical cut off is the full change at which the HIV RNA response first begins to decline. And the upper clinical cutoff is the full change above which a clinical clinically meaningful HIV RNA response and that means greater than 0.3 log is unlikely. And then, for changes that fall in the middle is that zone of intermediate response. And again, you know, we really want to choose drugs that are fully sensitive when we can talk when we concoct our next antiretroviral regimen. So how should clinical cut offs be used drugs with susceptibility below the lower clinical caught authors are most likely to provide virologic response, their preferred drugs for treatment regimens. Drugs with partial susceptibility, those are the only you go to them as a last resort, and then drugs, drugs with susceptibilities. above that. They're not likely to cause any virologic response, and they should not be used. I'm just going to briefly describe some of the genotypic phenotypic discordances where the genotype may say one thing and the phenotype may say another, why it occurs, and how it can inform treatment decision making. So the major reasons for these discordances are these mixtures. Also, there's incomplete phenotypic algorithms. So that AI programming, how to interpret those mutations may be incomplete, there may not be enough samples to really enhance that intelligence or algorithms, sensitivity. The improper weighting of mutations in algorithms also patients sometimes with non B subtypes. And again, sometimes these mutations can hyper sensitized re sensitize agents, and that Geno in and of itself, may not be able to detect that. So this is an example of discordant secondary to mixtures. And you want to note that the phenotypic bar graph appears to show susceptibility to most of the nucleosides. below the fold change Bar Bar Graph, you'll know that there are these mixtures within the genotype, for example, that M 41, M, slash L, et cetera. And underneath the evidence

of susceptibility, you know, that there's a lot of discordance between the genotype, and the phenotypes. And in most cases, again, we're going to side with the genotype if there are mixtures. But again, this net assessment then is going to factor all those things in and give you the correct answer. really supported by the latest data, and really allow you to pick the best drug for that patient. And again, these mixtures again, remember you need 40%, right? of M 184 MB mixture to be the V in order for that phenotype to be sensitive enough to actually correctly predict resistance. All right, so here's an example of resend solicitation in a discordance. Um, so again, we have where mutations here have actually hyper sensitized to drug. And even though on the genotype is saying no, when you factor in these discordance rules, the net assessment is that this drug is I WD, even in the presence of these mutations, is going to be sensitive. Okay, so again, and importantly to is we need this information sometimes, in order to make our best choice of next antiretroviral agents. I just wanted to give you this background, this historical background of how these tests are interpreted, but again, really, all you have to do Again, is this net assessment. So just to summarize, if you do see discordance between the genome and the fino,

45:09

if the phenotype says resistance and the genotype says sensitive, you're going to go with the phenotype. If the thing is sites have sensitive and the genotype says resistance, so you're going to look if there's mixtures present, the genotype is going to be more accurate, and if the mixtures are absent, is going to be the phenotype. And that's the basic rules for discordance. Now, we have a not so newer class of drugs, but the integrase strand transfer inhibitors. So we have primary mutations against this class. We also know that patients kept on a failing regimen ventric races will develop more secondary mutations, that may then start to impact the whole class. So again, if you're picking up true resistance, in the context of patients being adherence, draw your resistance testing really within a month, or sooner of the patient's stopping their meds, take off the pressure of the drugs, wait for these tests to come back. And then you can decide on what next AR T regimens to click. So, these are a listing of some of the primary and secondary mutations for some of the intergrates classes route Tegra, Vir L by chegar via which are which are consider lower genetic barriers. And then these are some for W Tegra. Vir and also big tech review. So the last testing that I wanted to describe is tropism and profile testing. So we know co receptor tropism is defined as the ability of a particular HIV virus to infect a target cell using a specific co receptor. So HIV requires two binding events to enter into a cell. It was first bind to the CD four, and then secondarily to a chemokine receptor either CCR five, or CX CR for less likely. So tropism is a label given to the virus that describes which chemo is taught which chemokine receptor the virus is using. So this is telling you what the hosts the patient's tropism is. So our five tropic viruses are our virus populations that can use only the CCR five dual tropic are viruses or virus populations that can use either the CCR five or CSC or for export tropic viruses are barred populations that can use only and then mix is viral populations that may contain various combinations of these. So all that you need to know is if you want to use the drug maraviroc, you gotta send for a profile test, and that that patient's virus has to be our five tropic only. Because if it's not, then this entry inhibitor is not going to be affected. So virus uses CCR five co receptors to enters the CD four cells. And that's the way that you would interpret that assay. The last test is a gene ashore archive, it's the newest. And it's a way to actually obtain a genotype in patients that have an undetectable viral load or low

level viremia. And it's designed to provide HIV antiretroviral drug resistance data when standard resistance testing cannot be performed due to inadequate plasma viral load. So if you don't have those 500 copies at least, or 1000. And you have low level viremia, or a patient is undetectable. Then this assay, interrogate the the reservoir, the archive, using next generation sequencing, to really extract pro viral DNA and then to be able to perform genotypic analysis. And we use this test sometimes if we're thinking about switches that we have to make them based on side effects, adverse events, regimen, some simplification, maybe we want change your regimen due to drug drug interactions, but we don't know if patients have had any, any archived mutations. And this is what one looks like. So it looks very much like that genie short prime. It lists the class generic brand. It gives you the mutation. And again, here again, this is an algorithmic fed interpretation assessment. It is not a phenotype. So that viral archive it's a second source resistance. Differences can exist between the viral population circulating the plasma and the pro viral DNA archived in infected cells.

50:12
So viral loads and standard resistance assays analyzed viral RNA but this gene ashore in Terry interrogates these archived HIV pro viral DNA embedded in host cells during viral replication. It's not a perfect assay. It's more specific than sensitive, so it may actually miss mutations. And that's more likely and patients that may have had HIV for long periods of time, decades. But if you see something on a genius short archive, it's likely to be real. Okay, so with that said, let's just move to our studio audience question. So here we go. Question number one, a 32 year old male newly diagnosed with HIV infection presents to your clinic for primary care. What HIV resistance test would you order? So brand new to the clinic, newly diagnosed with HIV? So what resistance tests would you order a genius short prime? You go for that fino sense? GT controlfile assay or all of the above? Very good. 71% Gina short prime right? Yeah, it's baseline. You don't really need to go to that fino since GT. We're not thinking of using Moranbah rock. So yes, correct. Initial prime Very good. Okay, the initial the initial resistance panel revealed wild type virus, and you decide to start patient on Jen Voya, which is TAF FTC. And then Kobe boosted L by Tegrity. Are patient does well and initially suppresses. But the last two viral loads are between 1002 1000 copies. CD four counts are stable. What do you do next? All right. So kind of confirmed that there's been two viral loads that have been detectable, CD four counts are stable. What are you going to do next? Are you going to know new chest? Just monitor patient and re stress adherence? Are you going to order a fina sense GT integrates plus profile, you're going to switch the patient to a PII based regimen or you're going to send a gene ashore prime 40% of the genome short prime That's correct. Again, here, the 40 cents go to a gene Tryphena sensitivity and integrates, I don't think even first failure, I think there's enough in the databank. There's enough match samples, you're probably just going to have one or two mutations that are going to be present. And again, I think the algorithm is good enough, the artificial intelligence is good enough that you don't have to go to the more expensive Venus sense GT. Nor do I think you have to consider using forever. Okay, so the last question here. Okay. So the genus short prime does reveal this single mutation, the M one ad for V, suggesting resistance to FTC only. So what do you do now? What do you recommend to the patient so you did the resistance test, it came back, he's off his gym boya Are you going to switch him to boosted darunavir FTC TAF or you're going to switch them to big Tegra veer FTC TAF or you're going to switch to big Tegra veer FTC tack plus directory or you're going to go to a dual arg

regimen of Doti Tegra Vir three TC and Okay, alright, so I'm interesting so but about 40% said switch to big tech have your FTC tab no one picked boosted darunavir so and said hey, we're gonna go heavy guns. We're gonna do big FTC tap enter every now and then third of the respondents said during your tenure, three TC so you know the correct answers I think in the presence of the M 184 be definitely a boost in PEI and big Tegra Vir would be affected.

54:26
What whether you choose one over the other could be based on a variety of reasons, but both of these would be effective because that M 184 V hyper sensitizes that to NOFA Vir. So you would maintain this mutation in the presence of the FTC and both of these would be effective. I don't think you would have to add a additional agent. I think with this early failure, this is one mutation I think this would be enough and then for W type revere three TC, we know that the M 184 B does knock out three TC And that that mutation doesn't hypertens hyper sensitized, doyou Tegra Veer, it's really not recommended that in the known presence of this mutation that you've switched to this dual and dual drug regimen, however, there is data to show that possibly even in the face of the M when ad for the that is to drug regimen may be effective, but I would say that for a more confident choice, it would either be one or two. Okay, great. Well, I just want to thank Dr. Deeks for some of these slides. And I will turn it over to Tara.

Tara 55:43
Great. Thanks so much, Dr. Bina, we're gonna go to questions. Our first one is, Can you recall again, how to test for integrase resistance?

55:54
Yeah, so the interface resistance is done automatically. If, if you order with a genotype, it's the genus, it's the genus short prime. And with a phenotype test, it's going to be the fino sense, g ci with enter grace, or there is one just called a phenotype with integrates. But I typically we get the finished sense GT with integrates. And that's how you could get integrates resistance testing on either genotypes of phenotypes.

Tara 56:24
Okay, great. We have another question here. How often should patients get a genotype and phenotype test?

56:30
Yeah, you know, not often now. And the great things about these newer drugs is that, you know, they're so good, whatever patients take, they're going to fully suppress. So really, you just do it at baseline before. And then it's only when a patient has detectable viremia that you feel is not due to poor adherence, if a patient is is virus is detectable because they're not taking their drug, then I don't think it's necessary to get a genotype just restrict adherence. So you would get a genotype phenotype resistance says only when you really feel that that patient has drug resistance, and typically, after two confirmed undetectable viral loads greater than 200. So that could be once a year, once every three years, you know, it could be very infrequent.

Tara 57:24

Great, thanks so much. Do you have any thing else to know about? That? Dr. Vino, with the CI line and no, yeah, kind of those inquiries?

57:33
Yeah, I would just say if if there's ever a patient in front of you, where you're, you're having you're unsure about whether you get a genotype or not, or a genotype or phenotype comes back and you're unclear, you can always call the CEI line. Remember, you don't have to make decisions quickly in terms of switching or new regimens. You can always take a patient off of their medications or just call call on the CDI line. And we'll help you with interpreting these tests giving you advice in terms of what next best steps to do and what next best AR T. There was one question here are their reflex viral loads to genotypic test like we have for hep C antibody reflect test? That's a great question. And no, we don't so there's no reflex testing. If it's detectable viremia that it'll automatically go to, to a resistance test. And just so that, you know, the the majority of patients that fail it's due because they're not taking their HIV meds and so I just wanted to end with showing everybody my email and you can e mail me any questions or concerns or just call them the CEI lab.

Tara 58:53
Great, thank you so much, Dr. Urbina, we're gonna wrap

[End Transcript]