Clinical Education Initiative
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HIV DRUG RESISTANCE INTERPRETING ASSAYS

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[video transcript]

[00:00:07] So, I'm going to talk about HIV drug resistance interpreting assays. These are my disclosures. So, the learning objectives are going to be to understand the various genotypic, phenotypic, and tropism assays to be run. Determine when to use combination resistance testing in patients with extensive antiretroviral experience. And to determine how to use archive testing, which is kind of a newer technology in patients with suppressed viral loads. So, some of the basics of antiretroviral management so, if a patient is newly diagnosed, new to treatment, then really all that you need to do is perform a genotype prior to initiating antiretroviral therapies. And you just want to make sure that they have what's called wild type virus and they don't have extensive mutations that may impact your initial regimen. Now, if your patient is in acute HIV infection or if you want to start prior to the results of the resistance testing, or I art or jumpstart, then you want to start with drugs that have a higher genetic barrier. So, either boosted protease inhibitor, and typically that's going to be boosted darunavir with either ritonavir or cobicistat, or dolutegravir which is like the protease of the integrase class. And then you want to combine them with either TDF or TAF with FTC. All right. So, for treatment experienced, those are patients that have had a history of being on HIV medications, it's best to get resistance testing within four weeks of them being on their meds. Because if it's more than four weeks, really more than eight weeks, then what happens at the wild type virus overgrowths any of these like mutant viruses and you're going to lose the sensitivity of this resistance testing.

[00:01:59] So, it's always best to get these tests when patients are failing on their regimen or within a month of them being taken off. That's because this wild type virus can outgrow these minor variants. So, we want to familiarize ourselves with the various resistance tests from monogram, and it's really the only go to lab that does resistance testing there are some homebrew ones at hospitals at like Department of Health. So, those are basically genotypic testing plus or minus including the intergrase, phenotypic testing plus or minus the intergrase, tropism assay, which I'm going to go over a little bit. Does the virus use the CCR 5 or the x 4 receptor in order to gain entry into the T cell? Because there are drugs that are active only in what's called R5-tropic viruses. And then archive genotypic testing is for those patients that are suppressed. Maybe they've been on and off meds in the past. You're unclear if they have any resistance. This is the testing technology that will allow you to do that. Okay. So, these are the test front monogram and we're just kind of going to go through them very briefly. So, the first one is this Phenosense GT and Phenosense GT plus integrase. So, what that combines is a genotype plus a phenotype.

[00:03:19] All right.

[00:03:21] And remember, a genotype is just amplifying the genes of interest that code for these enzymes, reverse transcriptase, protease, integrase. All right? So, it's basically PCR polymerase and you just amplify these genes and you see if there's any mutations. A phenotype, which I will show you later,

actually grows that patient's virus in a petri dish and then sees if increasing concentrations of drugs can inhibit the growth. So, it's a more kind of like powerful, I think, assay, the phenotype, but the Phenosense GT combines them both. Now there's just a phenotype which is just called the Phenosense. And then we have the genotypic testing. So, there's GenoSure Prime, that includes everything plus integrates and that's kind of the one that's really used, at least here in New York, a lot up front. All right?

[00:04:15] And then we have suppressive managements. Meaning if somebody is undetectable, and you want to know if they've had a history of ever having had mutations, you can do a GenoSure archive which will actually be able to amplify the reverse transcriptase, protease, and integrase genes. And then there's a trofile DNA which will allow you to establish tropism in people that are suppressed. Now regular tropism tests, meaning if they've got like a virus greater than 500, you can just do the regular trofile Assay. So, those are basically all of the tests. All right. So, this is kind of how a trofile Assay comes back. So, tropotype result, it's very easy to interpret, does this patient's virus have R5, dual mixed, or X4. And it's only if the virus is R5 tropic, can you use this entry inhibitor called Maraviroc. And that's how the report comes back. So, basically anything that's not R5 means that you can't use the drug Maraviroc. All right.

[00:05:27] So, some of the recommendations for resistance testing so the genotype testing is recommended at baseline. Genotypic testing is recommended at first and second failures, and then after that is when you really want to start including phenotypic testing, in addition to genotypic testing, that Phenosense GT. And make sure too, that you include the integrase, because you have to specifically ask for that Phenosense GT plus integrase. And then that trofile test you want to order, If you're thinking about maybe using this the drug Maraviroc in their salvage regiment.

[00:06:05] All right. So, a genotype identifies resistance when mutations are present only as mixtures, therefore they can give an early warning sign of let me make sure that's, yeah. They they can give an early warning sign that resistance is on the horizon. They're kind of like the the bear prints in the woods. You know I mean? So, they they happen early. When you see them they kind of predict resistance and at phenotype I'm going to show you how this Assay is done. You basically take out the genes of the patient's HIV virus, you transfect them into this murine vector, and then you basically grow the patient's virus and see if the drugs are active against it. So, it's a true test of like sensitivity. Both tests in combination may provide the most complete picture of actual resistance. And this approach is most useful in patients who are more treatment-experienced, like the case that we're about to present. So, here's GenoShore Archive technology. So, they extract PBMCs through DNA extraction, they take out the cellular DNA and then they do polymerase chain reaction to our HIV genes of interest. They amplify it and then they give you these mutations. And what's amazing about this is that any resistance in the life of the patient can be picked up in this Assaye.

[00:07:27] So, this GenoShore Archive, its sequences, the integrated proviral HIV DNA that's present in infected cells, and it can provide information about resistance associated mutations acquired through the lifetime of the patient. The circulating peripheral blood mononuclear cells are source of cellular proviral DNA that can be used for genotypic analysis. And this proviral DNA sequencing is can be a valuable tool when it's used in like looking at patients previous resistance test or also just their antiretroviral history. So, how well does it perform? I just want to show you here.

[00:08:04] This is a they were able to take samples where they could document previous resistance tests that had documented resistance patients, and subsequently suppressed, and they performed this GenoShore archive so that the sensitivity for all drugs was about 85 percent, not bad. So, it correlates very well with like actual genotypes and phenotypes. And it has a great negative predictive value, meaning that if you don't see a mutation it's really unlikely to have been there in the life of that patient's HIV history. So, and this is kind of what the report looks like. So, it's basically, it'll give you the mutations that were obtained using this cellular DNA extraction. One thing that's very important, and it also is germane to this case, is that a genotype whether it be a GenoShore archive or this one, it can only give you the mutations. And then what it does is that it puts it into this computer algorithm. All right? A little bit of artificial intelligence, but not quite. And then it can predict if a drug is going to be sensitive or resistant. But it's not a phenotype, it's not actually growing that patient's virus and telling you this, it's just that with these mutations plugged in then it's giving an algorithmic estimate if the drug is going to be sensitive or not. But to truly know you're going to have to do have a phenotype.

[00:09:36] So, why is it important that we had this information before we switch patients? Because if you have a patient that has a history of antiretroviral resistance, and if you now switch them to a drug that has a higher genetic barrier, and then all of a sudden they tell you hey I kind of want to go on a simple regimen or a once a day regimen. So, this study looked at patients that were on a booster protease inhibitor Kaletra two NRTI's. And they randomized them to either stay on this regimen or to switch to an intergrase based regimen that had a lower genetic barrier. And what happens is that when you switch to this regimen that contain raltegravir there was more virologic failures. Because these resistant mutations were not identified earlier, and patients were then randomized to this regimen and it wasn't enough to suppress the virus. So, it can have dire consequences. So, the PhenoSense GT. So, what it does is the PhenoSense GT takes out these genes and the HIV virus, gag and pol, and what it does is it then transfects them into this resistant test vector, and then it includes this little luciferase gene which lights up whenever the virus makes copies of itself. So, basically you take the patients test vector DNA because it lacks the gene envelope to make viral core proteins that can infect cells. You have to combine it with this murine envelope DNA, and then you basically combine that into a murine model and then the virus just starts to replicate. And then this little gene turns on when it makes copies and then you basically add in a petri dish then increasing concentrations of the drug. And then you get these curves. So, there's a patient curve and then there's a control curve, and the control is always like wild type virus; a virus that doesn't have any mutations.

[00:11:39] So, here we see that like the concentration to prevent to have 50 percent drug inhibition is the same in the patient as in the control. There is no drug resistance. If there is drug resistance to a certain class. Then what happens is that the curve shifts to the right, meaning you need more concentration of that drug to get that 50 percent inhibition. And those little whisker plots right there on the bottom kind of show you that.

[00:12:12] All right, and then sometimes though, just for complete sake, sometimes certain mutations can hyper-sensitize the virus against a drug and then the graph shifts to the left; you need less of the drug to get that 50 percent inhibition quotient. So, that's hyper-susceptibility and there have been trials to prove that. All right. So, this is what the PhenoSense GT looks like.

[00:12:37] So, this is a combined genotype.

[00:12:41] OK? And first what it does is that it lists all the drugs on the left, their brand name, and then these are already the cut offs that have been pre-determined by virology labs. All right? And then that full changes those graphs, those are the graphs where they've taken the patient's virus and done these curves. And that gives you the full change so that patients fold change versus wild type control. So, if that value was over the upper cut off and then that drug isn't going to work. Once it starts to reach that change and then you get these little hashtags. But then once it gets passed the upper limit, then the drug is completely resistant.

[00:13:26] So, those are how you interpret it.

[00:13:29] And then this net assessment then tells you, like combining the genotype and the phenotype, is this drug resistant or not. And this is this one is really the gold standard because a genotype would just give you those mutations. It plugs it into an algorithm, but does not really tell you how these individual mutations interact with each other, but this phenotype does. And then basically you just read the net assessment to determine which drugs are sensitive and which drugs are not and then the report kind of gives, puts out the list this way. So, that's basically resistance you know.

[00:14:09] And again, you don't have to make a move very quickly.

[00:14:14] You know what I mean? You can always obtain these tests and then reach out to a consultant and we have the CEI line where you can ask an expert about what's the best combination of tests and what's going to be the next best ARV regimen.

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