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Podcast Episode Title: An Introduction to Molecular Testing (what and when across the patient journey)

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Tonke de Jong
Welcome and thank you for listening to this podcast from COR2ED Independent Medical Education. In this episode, you will hear from internationally renowned experts, Professor David Hong, Medical Oncologist, and Dr Tracy Stockley, Pathologist and Geneticist, as they provide an introduction to molecular testing. They discuss when and what to test during the patient journey, the importance of multidisciplinary collaboration, how testing can differ depending on the type of setting in which you practice, as well as much more.

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David Hong
Hi, welcome to the podcast where we will be introducing molecular testing and considering when and what to test during the patient journey. I'm Dave Hong, I'm a medical oncologist and drug developer here at MD Anderson Cancer Center in Houston, Texas. And I'm joined today by my colleague, Dr. Tracy Stockley. Thanks for joining me Tracy. Perhaps you could give a quick introduction?

Tracy Stockley
Thanks, Dave. Happy to be here. So, I'm Tracy Stockley and I'm a clinical Lab Geneticist and I am head of the Division of Lab Genetics at University Health Network in Toronto, which includes Princess Margaret Cancer Center.

David Hong
Delighted that you can bring your pathology and genetics expertise to the discussion, Tracy. So, as we know, precision medicine oncology has been around for about 20 years now. HER2 in Breast, imatinib in CML, and the number of biomarkers in cancer types requiring molecular analysis has increased greatly during this period. We have now a huge number of therapies that are approved to treat cancers harbouring specific genomic biomarkers.

I'm just thinking about my case. I helped develop larotrectinib for NTRK fusion patients, also KRAS G12C and sotorasib. However, there's a lack of clarity as to when these tumour genomic sequencing should be ordered, what type of assay should be performed and how to interpret the results for the treatment selection. And on top of all that, the terminology alone can be very confusing. So hopefully we can bring some clarity in our discussion today.

So, let's kick off with some basics and clarify some terminology from the outset. We hear the terms genetic testing, genomic testing and next generation sequencing or NGS being used often interchangeably. Are these tests effectively the same?

Tracy Stockley
So, you know, just maybe starting with the idea of genetic versus genomic and the differences there. So generally, for me, when I think about genetic testing, I think it may be testing any kind of genetic variant. So, we do have tests that target only one or a few very common variants that can direct precision medicine up to maybe, some number of genes.

When we talk about genomics, for me, I feel that's something where we're looking at a much more extensive region of the genome. So, anything from, you know, whole genome sequencing all the way down to maybe even possibly very large target panels or things like transcriptomes that's looking at all of the expressed genes. So, there is to me some slight difference between those. And I guess it's really about how extensive the testing is. But they do tend to be used a little bit interchangeably when just talking about testing any kind of gene variant.

David Hong
I think another important point is, to highlight upfront, this difference between germline and somatic mutations. And so, what are the differences and how do you test for these Tracy?

Tracy Stockley
Again, some of this terminology certainly can be confusing. Just thinking now about germline and somatic. So, germline variants really are the genetic variants or the changes that you inherit from both of your parents. In theory, they're all the same in every cell of your body. Somatic are changes or mutations that you acquire in certain cells over time. And really those variants are limited to the cell they happen in or any descendant cells of that parent cell where the mutation was acquired. So that's typically what happens in a tumour. You might acquire some somatic changes that lead to the uncontrolled growth of the tumour.

And this also really impacts on the type of sample that you get. So, if you're trying to test for somatic variants, you really want to have access to the tissue in which those variants might occur. So, tumour tissue or anything that's related to the somatic changes. Whereas for germline, you can really test any cells in your body, but most typically just for ease of access, we would use blood samples and we collect the lymphocytes as the source of the germline for that.

David Hong
Tracy, there's a terminology called ctDNA. Can you explain what that terminology means and that testing, how is that different than actually testing from the tumour tissue?

Tracy Stockley
Yeah, and again, just thinking about some of the terms we use. So, there is ctDNA but also cfDNA that you might have heard. So cfDNA is cell free DNA. It's just any DNA fragments that exists outside of cells. So, typically in body fluids. So, we can collect that from the non-cellular parts of blood or even from lots of other fluids like pleural fluid or from urine. And a fraction of that cell free DNA can come from a solid tumour that the patient might have. So, we call that the ctDNA or circulating tumour DNA. So, they are related, but just slightly different.

So, when we're trying to look for circulating tumour DNA and cell free DNA, we're collecting non-cellular parts again, typically we use blood samples and we would spin down any of the cellular parts and collect the cell free DNA from the plasma. And the cell free DNA, again, trying to look for any tumour markers that are occurring there. That can just be an easy way to sample for solid tumours, especially if it's difficult to go in and actually get tissue for a patient or if the time to getting a biopsy is a problem and you need a really quick answer for that patient.

So, when we look at variants that we find in cell free DNA, we also can relate that back to the variants that we see in the tumour tissue. So, these are not always perfectly correlated. We would expect if a tumour tissue has a variant that we would be able to find that in the ctDNA. But that is not always the case. Sometimes that tumour isn't shedding cells that provide access to material as ctDNA and sometimes we detect things in ctDNA that are not found in tumour. Just, there we're thinking maybe that's because of tumour heterogeneity or sampling errors.

So, Dave, let me ask you now, how and when are you using ctDNA in your clinical practice?

David Hong
I think really a lot of it is just ease of use, just logistics. Ideally, if I can get tumour tissue NGS, next gen sequencing, ideally whether from a recent biopsy or recent archived tissue, I prefer that, as you mentioned, partly because there's sometimes can be discrepancies between the ctDNA and tumour tissue. But oftentimes that's not possible. Situations where biopsies are difficult to get. Biopsies are not benign procedures. And/or there are certain tumour types that sometimes obtaining lots of tissue can be difficult, whether it's pancreatic, sometimes we get FNA for diagnosis, or lung which also has a high risk of complications from a biopsy.

And secondly, usually the turnaround time for tumour tissue genetic testing NGS can be prohibitive in the sense that depending on, I guess, the assay or your centre or the send out, it can take up to two weeks or sometimes even more. And you really want a faster turnaround time to make a decision about a patient's care. So ctDNA as you know, usually that turnaround time can be much quicker.

So, I would say those are really the two major reasons. The other reason, which is more for research purposes and we do that a lot here in our department, is that oftentimes with these new targeted agents, patients will progress. And we want a quick read on, from research perspective, what resistant mutations are arising, is there, at least in our department, another trial that we can try to get them on, etc.?

Tracy Stockley
Yeah, thanks. I think those are excellent points and certainly, you know, from the lab side having access to ctDNA as an alternate or additional sample, especially as you say it at progression is very important.

So maybe just thinking back then just to molecular testing in general, are you testing for germline and somatic mutations in all of your patients?

David Hong
So, we are mostly as somatic mutations arise, we do some kind of molecular testing in all of our patients, or we try to. Partly because, obviously we believe in the value of these mutations, etc. And the type of patients I see in our clinic are mostly metastatic chemo-refractory patients who may have already had some type of testing.

In routine clinical practice obviously, you want to get germline and somatic mutations, in my opinion, as early as possible, particularly in the metastatic setting. Germline testing really in the context of family history, right? I mean, obviously as physicians, we need to get a good history and a family history is obviously very important. Had there been close family members who also had similar cancers? Is there a family loss, any family history? There are certain mutations that, as you know, that can arise in somatic mutation analysis like *BRCA*, MSI-High and other alterations that may suggest that the patients may have germline mutations.

I think one important point with germline mutations is obviously that not only affects that patient, but it may affect their immediate family members and beyond. So, it's important that if you do get germline testing and there's something that arises or you suspect it then you want to consult genetic counselling in that context.

Tracy Stockley
Yeah, that's an excellent point, the ability to identify family members hopefully before the onset of any cancers is very important and just helps them have a better outcome overall.

Just again, thinking about the testing you're doing, are you testing right at the point of them first seeing you? Do you also test at progression? So just where in the patient journey are you testing?

David Hong
That's a good question. My clinic is a little specialised in the sense that again, most of my patients have been sent here for a clinical trial. That may be unique to, I think, academic centres like MD Anderson. But we do try to get some type of molecular testing or they will have come in with some type of molecular testing from the very outset. I know my colleagues in the other tumour specific departments, particularly thoracic, will try their best to get molecular testing, whether it's tumour NGS or ctDNA, from the very outset, because that may ultimately affect obviously what type of therapies these patients will be receiving.

We do our best here from a research perspective to really try to obtain progression samples, whether it's from another biopsy or ctDNA, to really understand why these patients are progressing, but also think about next therapies, maybe next trials that they may be open to. I'm seeing here in the institution patients who are getting tested earlier and earlier in their course, partly because we now have these drugs that can be really actionable from the outset. So even in the maybe stage three or adjuvant setting, I've seen patients actually get NGS testing in the event that they again progress and then they can be quickly transitioned to a targeted therapy or so forth.

So I don't think there's one specific pathway, but particularly in the metastatic setting, I'm a big believer that now that we have so many different tumour agnostic therapies, whether it's drugs that target NTRK, like larotrectinib and entrectinib, I mean, there are now, as you know, tumour agnostic therapies for RET and BRAF, etc., that it's important in the metastatic setting to get that next gen sequencing testing done because it would open up additional opportunities for therapies that these patients may not have gotten in their typical lines of therapy for their specific tumour.

Tracy Stockley
Yeah, that's a great point. From the lab side, I certainly feel that when the tumour, the first tumour agnostic indications came out, it really didn't change a lot of how we thought about some of the testing that we're doing targeted versus more extensive testing.

David Hong
Okay Tracy, I think one area that's perhaps confusing for a lot of physicians and oncologists is what assay to order and should we be performing multi gene panel assays for all patients or requesting specific biomarker tests in certain instances?

Tracy Stockley
Over the years we certainly have many more genetic tests that we can apply for precision medicine, and it does get, you know, a little complex as to what to use when. So maybe I'll start with, sort of, the simplest and usually the fastest, which are just looking at a small number of variants typically in what we call hotspot mutations or even cytogenetic things like FISH for copy number changes. So those tests, because they're fairly limited in scope, can often be very quickly done. And so, it might depend just on the presentation that patient or the particular point you're at, whether you might want a test like that.

And then the next step would be something more extensive. So usually now this would be using next generation sequencing. So next generation sequencing is just our ability to look at multiple genes all simultaneously. And we can really have large footprints when we do this on next generation sequencing. So, for example, one of the panels that we run at our site is a 500 plus gene panel that looks at both DNA and RNA for fusion. So very comprehensive. So the challenge there is that NGS does still, because it's a multi-step process, it does still take more time. And, you know, sometimes there is a need for a higher quality of sample when you're trying to do something very extensive like that.

Just thinking then about the treatment choice. So the real advantage of doing something where you're getting multiple genes or multiple information at once is that it might help you just in selecting your treatment because you have a large amount of information to look from versus hot spots where you're really just saying, does it have the most common things I might expect in this tumour?

And finally, I think the other challenge would just be the access to this. So NGS, especially really comprehensive things, are maybe only available through large academic centres or through some of the private lab vendors, whereas community labs or others who don't have access to that kind of testing may not be able to have that information quite as easily.

And so, David, I'm also interested to hear how you might work with your lab and the pathologists at your centre, especially to interpret results of the test and determine what might be actionable? Especially when we start to think about larger NGS panels, the data can be complex and so I'm curious, what elements you consider when reviewing these kind of test results?

David Hong
Yeah, Tracy, thanks for that question. Pathologists are so key and at least what we do here in our department, but I know for other oncologists and oncologists outside of our department and we utilise our molecular pathologists and there's a subset of molecular pathologists that are kind of linked to a lot of what we do called the Institute for Personalised Cancer Therapy. And as you know, with NGS, there's not only hundreds of genes and mutations, somatic mutations may arise, but there are like hundreds and

thousands of variants within specific subsets of genes. And so we really depend on them to help us annotate these mutations and variants to what is really out there, to suggest that this is truly actionable.

For example, we have a very large multidisciplinary review meeting, we'll present our patients, most of them, if they have it, will review their NGS or panel and review that in length to see which of these mutations that are arising are truly what's called actionable. Right? Something that has been in the literature or has been known to be a target or a region that we can target with a trial or known standard of care therapy. And so, they've been really instrumental in that obviously, they've been instrumental in how we do research here at MD Anderson.

I think you bring up something which is important and that is that community hospitals oftentimes cannot do this NGS testing, partly because of not only the complexity of NGS, but also the bioinformatics that is needed on the back end to generate these reports and a lot of community pathology groups really just depend upon the reports that an oncologist sent to them by Foundation, Tempus or these vendors out there? But there is, I think, incredible value in a multidisciplinary review, questions that sometimes you can't ask the reports. So, we really depend on the local pathologists to help us make directions about how to treat our patients.

Tracy Stockley
Yeah, thanks. I think it's probably similar for us, especially at Princess Margaret. We do have multidisciplinary teams and reviews that are site specific, and I think have tremendous value, but our lab especially, we also act as a centralised lab for other patients from across our province or across Canada.

And I think you mentioned about the reports, I do think we very strongly feel it's a very important part of the lab role to make sure that our reports are comprehensive, can be really informative to people who are not maybe at a major academic centre and provides enough information on the variants and their clinical significance that the oncologists knows what to do once they get that report.

We also do have some reach out programs, especially with Princess Margaret, where they interact with other community hospitals to provide extra support for all aspects of care, but also including the genetic tests that we participate in. And I think when oncologists are thinking about sending out samples for testing to labs, probably across the US and certainly in Canada, there is definitely choice of where those samples can be sent. And I think that is an important part, just how does the lab support you with the reports, with their own information? Do they have databases of the things they've seen before that helps them interpret new things? And all of these things really become important when you start to think about outsource testing.

So definitely there are pros and cons of having in-house versus outsourced testing both in technology and, as you say, a bioinformatics expertise available versus the speed of getting a result if it's right within your own hospital.

David Hong
One of the surprising things I think has happened since the development in NGS is how, at least here in the United States, we haven't been able to scale it as widely and rapidly as initially thought it could go. It is much more common now here in the United States, but I think for a while it was surprising to me that it took so long, given its value. But I think a lot of these kind of logistical issues, whether it's reimbursement or in the community hospital where you just can't get NGS in-house, you really have to send it out. All these other issues really kind of have somewhat delayed it.

I'm assuming we're going to at some point figure out some of these kind of hurdles down the line. But there's still some challenges. When I think about some of the drugs that I help develop, whether it's larotrectinib, which is a miraculous drug, if you're able to get larotrectinib or entrectinib for NTRK fusion patient, and you're able to identify those patients with NTRK fusion, it can be a drug that can be lifesaving, one that can keep these patients alive for long periods of time. Or some of these new drugs, such as sotorasib in KRAS G12C. I think that this is something that community hospitals, community pathologists, community oncologists really need to think about, really doing their best to try to get NGS or testing for their patients upfront. I hope, but I do think that at some point we will really be able to get widespread scaling of NGS into the community.

Tracy Stockley
Like you say, some of these drugs are just phenomenal how well they work and just the new access that patients have for these treatments is amazing. It's similar, it seems from the lab side, that maybe in the early days of NGS it was hard to get a good quality of tissue, the right amount of tissue to be able to do NGS. Over time, the amount you need and the ability to work with sort of less ideal samples is improving. So certainly our fail rate on NGS is much less than it had been at the beginning, and I feel it gets better all the time.

One of the other challenges I feel we are probably experiencing on lab side is just access to appropriately qualified people, both at the lab technologist level, the pathology level. The training systems are not producing enough people for us to have all the people we would like to staff this and make sure that we get all the turnaround time done quickly.

But, like you say, I think certainly it is improving and it might just be a little bit more time until we have sort of the kind of widespread testing that we all would like to see.

David Hong
Well, Tracy, thank you again for getting on this podcast with me. I think we could carry on talking about this topic all day, but we probably need to wrap up. So really to summarise some of our thoughts today, we talked a lot about the different technologies in testing for both somatic and germline mutations. We talked about the differences in the definitions of those, what the differences are between tumour next gen sequencing versus something like ctDNA. We talked a lot about when we should consider testing these patients with these platforms in the journey of these patients. We talked about some of the challenges I think that we all experience here, but also for our community colleagues. And we talked about the potential, I think, of some of these new therapies that can target these specific mutations if we are able to identify them.

So, thank you again and thank you to our listeners. We hope you've enjoyed our discussion.

Tracy Stockley
Thank you.

Tonke de Jong
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