Podcast Transcript
Perseverance pays off - Looking beyond the obvious mutations in non-small cell lung cancer

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Tonke de Jong (COR2ED)
Identifying targetable alterations through molecular profiling is a key part of today's treatment strategy for many tumour types, including non-small cell lung cancer. Keep listening to find out more about the importance of persevering with molecular testing to identify actionable mutations and to hear which tests may lead to some of these alterations being missed.
Thanks for listening to this podcast episode from COR2ED independent medical education. This episode is supported by an independent educational grant from Bayer.
So, today's topic is all about perseverance pays off, looking beyond the obvious mutations in non-small cell lung cancer. I'm honoured to introduce to you to two experts in the field of precision oncology. Professor Frédérique Penault-Llorca, Pathologist and Professor Mark Socinski, Medical Oncologist. We're very excited to listen to your discussion.

Prof. Mark Socinski
Hello and welcome to today's podcast where we're going to talk about a very important topic. Perseverance pays off - looking beyond the obvious mutations in non-small cell lung cancer. I am Doctor Mark Socinski and I'm the executive medical director of the

AdventHealth Cancer Institute in Orlando, Florida. I've been a lifelong thoracic medical oncologist, and I still am active in practice and clinical investigation. I'm extremely delighted today to be joined by Frédérique Penault-Llorca. Frédérique, would you like to give yourself a quick introduction?

Prof. Frédérique Penault-Llorca
Sure. Thank you, Mark. Great to be here. I'm a pathologist and a molecular pathologist. I work in the Comprehensive Cancer Centre in Clermont-Ferrand, in France. And I am involved in lung cancer diagnosis and molecular testing.

Prof. Mark Socinski
Thank you. So, let's get started. You know, we're celebrating the 20-year anniversary of the first report of *EGFR* mutations and now they've certainly become very important in the management of non-small cell lung cancer. But we now have 9 or 10 others, what we would refer to as oncogenic drivers, including five mutations (I mentioned *EGFR*, but certainly *HER*2, *MET*, *BRAF* and *KRAS* mutations are relevant) and four fusions (*ALK*, *ROS*1, *RET* and *NTRK*). At least in the United States, and I think probably throughout most of Europe, we have multiple targeted therapies that are highly effective in many of these subsets of non-small cell lung cancer. However, you cannot use these highly effective therapies unless you make the molecular diagnosis. And so, part of the diagnosis, as I always say, is what the molecular profile is. So, there's lots of possibilities for patients and our job as medical oncologist is to get the right treatment to the right patient at the right time. And so, we rely on our molecular pathologist to do comprehensive genomic testing. This is the standard of care. We do know that testing rates are not where they should be and there are lots of different reasons and barriers that we have to overcome; sometimes it's an educational barrier, sometimes it's a tissue barrier. We also know that there are some tricky ones that may escape being detected, such as fusions. And so, Frédérique from the molecular pathology point of view, what should we, as clinicians, be informed of in terms of performing these molecular tests, and what do you think is the best way to make sure that we never miss one of these oncogenic drivers?

Prof. Frédérique Penault-Llorca
Thank you, Mark. First of all, I think that before talking about molecular testing, I think there is a major point to consider, which is don't test too late in the course of disease. I think it's very important for the patient, and also to ensure appropriate molecular testing can be conducted. First of all, for the clinician, is to be in a position to send us sufficient tumour material, because especially in lung cancer, tissue is the issue. So these samples may be bronchial or transthoracic biopsies, biopsies of metastatic sites, cytology samples like brushing, EBUS, pleural fluid or node aspirates. They can be very rich in tumour cells. We can also perform this molecular testing on liquid biopsy, that could offer a more practical alternative when accessibility to tissue may not be possible for genomic alteration screening. But always keep in mind that we need to be sure that it's a primary lung tumour - so to exclude metastasis - so we need the tissue. And we also need tissue at one moment to assess PD-L1 because we need to have the tumour cells to assess PD-L1. Also, genomic testing is sometimes performed in a series of single gene tests. A more comprehensive multi-channel testing approach is highly recommended. And in particular, next generation sequencing (NGS), for molecular testing. And today it's agreed that NGS should be preferable

to individual single gene tests to ensure a thorough evaluation for multiple biomarkers in one single assay to avoid tissue exhaustion. And I would say, furthermore, NGS testing should ideally cover both DNA and RNA, as assessing DNA alone does not capture all the gene fusions, and this is essential for *NTRK* fusions, but also for *ALK*, *RET* and *ROS*1.

Prof. Mark Socinski
Yeah, and I'll reinforce the point you made about testing at the time of initial diagnosis. Again, I think it's part of the diagnosis that we see. It's not okay for me to hear that a patient has adenocarcinoma of the lung. I need to know whether or not they have one of these 9 or 10 biomarkers, including PD-L1, which you mentioned there. You know, one of the things that really has transformed my practice is the use of liquid biopsy or plasma-based testing. It has great clinical utility. I view it as being complementary to tissue-based testing. And as you well know, Frédérique, you often are faced with limited tissue in this disease, so liquid biopsy can be helpful. If you find something in liquid, you can act on it clinically. The issue is the false negative rate of the liquid biopsy. And you know, we still struggle with the volume of tumour. If it's a low volume state are you going to get a false negative. So those issues need to be known by the average clinician, because we have the opportunity to find things in tissue that might not have been picked up in blood. So, there is a rate, I think the most recent numbers I've seen is about 35% to 40% of patients who have an initial negative liquid biopsy can have an oncogenic driver found in tissue if there's adequate sampling there.

Prof. Frédérique Penault-Llorca
Yes. That's what my experience is too. Do you perform liquid biopsy at progression?

Prof. Mark Socinski
Yes. And I was getting there too. I think that's a very good point because we are beginning to understand in certain clinical situations what the acquired resistance patterns are and sometimes retesting can be informative. And I generally do start with a liquid biopsy because that's easy. The patient is in clinic, we generally have the kits there, we can draw blood and send it off. The turnaround time is about a week or so. So, if we don't get information or if you suspect there's been histologic transformation, that would be a reason to get a tissue biopsy. But probably the most common thing that I see in clinic is in a patient with an *EGFR* mutation, one of the classic mutations, that's been on treatment typically with osimertinib for a period of time and at the time of progression, they have high level *MET* amplification. And that's an informative retesting, whether it's in tissue or whether it's in blood. That is something that we would act differently on, perhaps adding a *MET* inhibitor before we moved on to chemotherapy and other things. So that's important. The issue that you raised too I want to emphasise, and that is the use of DNA and RNA, particularly as it relates to the detection of fusions. Could you just reinforce that, Frédérique, in terms of the importance of that?

Prof. Frédérique Penault-Llorca
Yeah. In fact, when we want to look at fusions, we can sometimes use immunohistochemistry. That could be indicated, especially for *ALK*. When we have a high expression of *ALK* we can use in-situ hybridisation (FISH) that doesn't give us the partner

gene, only that we have a translocation, a rearrangement of the gene. So, for *ALK*, *ROS*1, and *RET* we can use FISH. It's not always extremely sensitive. It's consuming a lot of tissue. So, we usually use, NGS. The problem is that some of those fusions, when there is a rearrangement, it can be either in the exon or in the introns. And, when you are using DNA, you are only looking to the coding area, so the exon. So, if you have a fusion in an intron, you can miss it when you use NGS with DNA based. So that's why for fusion I really insist that the best test will be to use RNA based NGS. Because with that you will capture all the fusions and you will not miss any fusion involving introns. And this is particularly important in some *NTRK* genes, especially *NTRK*2 and *NTRK*3 where most of the fusions will involve introns.

Prof. Mark Socinski
And could you comment on the use of PCR based tests and the pros and cons of PCR based testing?

Prof. Frédérique Penault-Llorca
Yes. Well, there are a lot of barriers for the use of NGS. And most of the time the barrier is economic. But, in fact, we have to be aware that the final cost of accumulating several single gene tests could be at the end extremely, extremely high. And also, we have a risk of tissue exhaustion. And in fact, today, when we look at the number of targets that need to be explored, 9 or 10, and if we carry out immunohistochemistry for *ALK*, *ROS1*, *NTRK* and *MET,* and FISH to confirm for other genes, as I said, and then PCR for *EGFR*, *KRAS*, *BRAF,* at the end not only can we end up with completely worn out tissue, but also we've techniques that added together will cost as much as NGS, but also with a lower sensitivity, because those PCR tests they cannot cover all the alterations, so we can miss all the rare alterations. Also, the complex alterations are frequently missed. And, for instance, if we look at the comprehensive popular PCR panel, they will miss some of the rare targetable *EGFR* mutations, such as some mutations in exon 18, in exon 21, some insertions in exon20. And we have targeted therapies for that, they can be targetable. So, if we use those PCR panels, we can really miss some of those alterations. And they account altogether for 15% of *EGFR* alterations - so, it's not a small number of patients, it's quite a large number at the end of the day.

Prof. Mark Socinski
Yeah, I would agree, I think you know perseverance is the key here. I will tell you a case that we had in our practice lately was a lady who had been about five years into her disease. She was a never smoker. She had been tested back around 2016-17. She did not have an identifiable alteration at that point. She got about four lines of therapy. She responded extremely well to pemetrexed, and that lasted a long time. She started to fail and we were having a discussion about, you know, what to do next. Should we continue treatment, or should we pursue supportive care? She actually discussed hospice with us. We sent a liquid biopsy, just to make sure in this never smoker that we had covered all the bases. And she ended up having an *ALK* fusion that was detected on liquid biopsy. And here was a lady who had heavy disease burden in her liver. She was progressing in her lungs. She was losing weight. You know, she was thinking that she was at the end of her disease course. And we started *ALK*-based therapy and she's had essentially a complete response. And now it's been a couple of years. And here's an example of how you can change the prognosis of a patient who thinks that they're at the end of their life. But this lady, based on the data we have with

the *ALK* inhibitors, may live another five, six, seven years with *ALK*-based therapy. So, I think, continuing to have a clinical suspicion in patients who might initially be tested negatively and making sure that you've done optimal testing. As you make the case for both DNA and RNA based NGS, consider plasma testing at that point and don't give in to this, what I hear a lot from my colleagues is, 'testing fatigue', if you will. You know, I hear, oncologists say, who don't necessarily focus on lung cancer, 'you know I test, I seem to test all my patients, but I don't find anything and therefore I get frustrated' and this sort of thing. And I keep saying 'keep testing, because sooner or later, you're going to find something that's going to make a big, big difference in the life of the patient'. And this is a great example. This lady is now probably 2, or 2 plus years on alectinib. Her liver has had a complete response, her lung also. She's obviously eternally grateful. And her family, who thought they were losing mom, are really grateful because mom's going to be around for a lot longer than they thought she was. So, I think that this is a very nice example where perseverance pays off in the end and we were fortunate to find that for her. But what are your thoughts about this issue of testing fatigue at this point?

Prof. Frédérique Penault-Llorca
Yes well, I often hear about this testing fatigue, but I really think it's very important to persevere. And I had this experience at the beginning also with *NTRK* alteration, not only in lung but in many other tumour types. But I think it's also important to persevere, even if we have depleting samples. We had a case of a woman; she was 56 with mucinous stage four lung cancer. We had the biopsy, and she was difficult to biopsy. She was a former smoker, and we had biopsy with only 30 cells available for analysis. So, we tried liquid biopsy, but the liquid biopsy was negative and probably her tumour was not shedding very much and she was progressing. And we said, 'well, we should try NGS', and we micro-dissected the biopsy, we performed NGS and we found a G12C mutation. The patient was treated with a G12C inhibitor, and she had a good response so far, it has been like 6 or 7 months now. So, we persevered, we succeeded, and this was for the benefit of the patient. So, I think that we have almost 50% of our lung cancer patients with possible targeted treatment. So, we are not allowed to stop. We must persevere.

Prof. Mark Socinski
I completely agree with you. I was going to say that same number. If you look at if you add up, the *EGFR* mutants *ALK, ROS*1, all ten of them together, including *KRAS* G12C it might be a little better than 50% that you're going to find something. You know, in the case of *KRAS* G12C, this is a second-line treatment. But some of those patients do very well with treatments and it's important to know that at the time of diagnosis. So, we've uncovered things like that in patients who come to us for second opinions. And we see that their initial testing may be PCR based. We've had a couple of *EGFR* exon 20 insertions that have been missed by PCR based testing and we've detected when we've repeated next generation sequencing. And so, I think it's very important, as you point out, that you persevere. And again, given the fact that I think slightly more than 50% of patients with adenocarcinoma are going find something, that testing fatigue shouldn't exist anymore, because you're going to find stuff in at least half of these patients. And so, I think that really is the argument to continue going on. And then, you know, I think as we move forward in understanding about resistance mechanisms, I think retesting will also play a larger role as we understand more of

this in future generations, of these targeted therapies and that sort of thing. What is the biggest challenge from your perspective, Frédérique, in the molecular pathology world?

Prof. Frédérique Penault-Llorca
Today, if we talk about, and we focus in on lung cancer, it's really the tissue availability because most of the time we have very small biopsies, and sometimes we receive, samples from other institutions where they have already performed immunohistochemistry, and sometimes some immunohistochemistry also for diagnosis that were not fully required. And we receive exhausted tissue or partially exhausted tissue. So, for me, this is really the biggest challenge is to have enough tissue and also to have access to the good techniques to be sure that we cover all the alterations that could be targetable for the patient. But it's really for me, the tissue, and we are doing a lot of education for pathologists to explain to them how to save the tissue, how to sample the biopsy in different blocks, how they trained the technicians and everything, to be sure that we have enough tissue for the diagnosis and then for the molecular testing. But as you said, now we have also the liquid biopsy. So, it's really a very good alternative. And, if at the end of the day, the liquid biopsy is negative, we have to go back to the tissue. So, we should re-biopsy the patient even if we don't have tissue because we need to give a chance to the patient.

Prof. Mark Socinski
Yeah, I completely agree. We have spent a great deal of time in our thoracic tumour boards, we have them weekly, educating our pulmonologist, our thoracic surgeons, our interventional radiologists about what our needs are. We are very collaborative - and I would encourage all the oncologists listening to this to make sure you know your pathologist and your molecular pathologist and have an intimate, professional relationship with them. So, everyone knows that, because it's a team approach, right? We all have to work together to make sure that we get the diagnosis in, and in 2024 the diagnosis includes NGS based testing to make sure you're not missing any of these oncogenic drivers where there's, in most cases, highly effective targeted therapies that will help patients much, much better than chemo, much, much better than immunotherapy. In many of these subsets, immunotherapy is not very effective from a clinical point of view. And, you know, something much to the credit of our tissue procurers, they've done actually a good job of making sure that initial biopsy, whether it's a core, whether it's a FNA or whatever, we don't necessarily see what we might have seen, you know, 7 to 10 years ago - quantity not sufficient for testing, that's getting less and less common. And to your point, you also made the point about the role of liquid biopsy that really has been critical in terms of the practice of thoracic oncology.

Frédérique, I want to get your opinion on one thing, because many oncologists ask about this. Does your institution do reflex testing?

Prof. Frédérique Penault-Llorca
So, in my institution, we do reflex testing for our patients. I think that this has several advantages. First of all, we have the sample, we make the diagnosis, and right away we refer to the molecular biology platform. So, there is no tissue exhaustion. We don't go back to the block, and we can save tissue, but also we can give the results very early with the pathology report. So, from the moment when we know that it's truly primary lung adenocarcinoma, we

will go to the reflex testing. I think it's really very good for our workflow, for the multidisciplinary tumour board, and it doesn't delay the patient treatment.

Prof. Mark Socinski
How do you deal with - because I've heard some pathologists, at least in the United States, say they don't necessarily know what the stage is. And so even some of our pathologists will not reflex the tissue because they aren't quite sure of the stage. And obviously, you could make the argument in early-stage disease that really the only thing that we care about clinically is *EGFR* and *ALK* at this point. Your thoughts on that comment?

Prof. Frédérique Penault-Llorca
Well, this is all about communication. In fact, we have a multidisciplinary tumour board for all of the biopsies that are going to be performed in the hospital. So, we have transmission forms with the indication, and for lung, we have indication, whether the patient is a smoker or non-smoker, it will be very important if the patient has a squamous carcinoma but is a non-smoker and, also, we have information of the stage. But anyway, as we, in my institution, have decided to test very early now even if the patient is at an early stage with a non-advanced disease, we will perform the molecular testing.

Prof. Mark Socinski
And from my perspective on the clinical side of it, I really like reflexive testing because it starts the clock of testing faster. And we're going to get the results a little bit quicker versus by the time the patient sees me, if I have to request it, we've already lost a week or two because of scheduling issues and these sorts of things. So again, this is another example. All of you listening out there need to get together with your pathologist and talk about what the issues may be. And if you can, reflex as many of these cases as possible.

Prof. Mark Socinski
Frédérique, this has been a great discussion. Obviously a pertinent and interesting topic for those of us who care for lung cancer. I want to thank you for sharing your insights. I'd like you to give our listeners what your key take home messages are.

Prof. Frédérique Penault-Llorca
Well, my key take home message is, don't test your patient too late, talk to your pathologists, and it's really a multidisciplinary concern. Make sure that the right tests are carried out. So NGS - DNA, RNA - because from a medical and economic point of view, this is a truly cost-effective option that can benefit the maximum number of patients with the appropriate targeted treatments. And last, but not least, pursue molecular investigation if the initial single test is negative because perseverance pays off.

Prof. Mark Socinski
I could not agree with you more. You know, one of the more enjoyable professional relationships I have is with our pathologist, because they're very helpful and for us clinicians, we really can't do anything unless we have excellence in pathology to direct us. And obviously, nowadays, a big part of the pathologic workup of these patients is the molecular aspect of it. So, I want to thank you again, Frédérique. And thanks to our listeners. I hope you enjoyed our discussion today.

Tonke de Jong (COR2ED)
Thank you so much for sharing these insights Professor Penault-Llorca and Professor Socinski. We've learned a lot from your discussion on perseverance pays off, looking beyond the obvious mutations in non-small cell lung cancer.
If you liked this episode and want to find out more on precision oncology, then look on the 'Oncology Medical Conversation Podcast' under the account of COR2ED medical education for other interesting episodes. Also don't forget to rate this episode, subscribe to our channel or inform your colleagues about it. Thank you for listening and see you next time.
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