[Voiceover] Welcome to The Pathologist Cut podcast. This RCPA podcast highlights the critical work of pathologists and the integral part pathology plays in medicine and health care.

[Dr Michael Dray] Professor Sandra O'Toole currently divides her time between Royal Prince Alfred Hospital, where she is a senior staff specialist in the Department of Tissue Pathology and Diagnostic Oncology. And as a researcher at the Garvan Institute of Medical Research. So if we can talk a little bit about breast cancer in general, we know it's the most commonly diagnosed cancer in Australia and it's up there in New Zealand as well. What have you seen happening with cancer rates in recent times? And how do you think research is impacting on that?

[Prof Sandra O'Toole] Research has had a huge impact on breast cancer. In fact, breast cancer has been the poster child for the power of personalized medicine. So it was way ahead of the curve. One of the first cancers that had these really smart, targeted treatments that came about through an understanding of basic biology and, in fact, development.

So if you actually look back and there's this fascinating paper in The Lancet in the late 1890s, I think it was, and Beatson, who the Beatson Institute in Glasgow was named after he was a breast surgeon. And when he was a medical student or even before he was doing some research into lactation and sheep of all things. And made some observation that by taking out the ovaries, that there were changes in the mammary gland such that it involuted you know, that makes a lot of sense, too. We know that, you know, from human pregnancies, obviously there are huge changes in the breast and obviously the same in all other mammals as well. So he took that fundamental observation that if you cut out the ovaries in a lactating shape, you get involution of the gland. And that just suddenly got him thinking. He had a series of patients who present with advanced breast cancer.

And if you imagine back then there were very few, if any, treatments for patients who presented with advanced breast cancer. But he thought back to his studies in sheep as a young student and wondered if he did. And you did an ovariectomy and that's taking out the ovaries of these patients with these big, advanced breast cancers.

What might happen? Because he hypothesised something in the ovary, he put it might be irritating the breasts. And we now know, obviously, that, you know, estrogen and progesterone are being produced. And that has a huge impact on normal breast development and in cancer.

And so remarkably, he did a series of patients operations and found that some patients had total resolution, at least temporarily, of these advanced breast cancers. So I think that was the first really great breakthrough in understanding between basic physiology, basic understanding of cellular processes, and that then coming up with an idea of how that may relate to cancer and then devising a treatment based on that big gap then for a

 

long time. But then there was more research understanding the role of the hormones. And in fact, a group of researchers who were trying to develop the oral contraceptive pill found an anti-estrogenic agent that was tamoxifen, basically related tamoxifen, which we know now.

It's one of the very common drugs used in breast cancer to block hormone positive breast cancers with incredible outcome success in terms of reducing deaths from breast cancer. But it took this failed contraceptive pill and then further research to develop one of the most effective treatments for the targeted specifically to breast cancer or, you know, really targeted at the time to any sort of cancer that we understood. And specifically in breast cancer, by blocking hormones, were able to slow the growth of tumours. And in fact, prevent progression of a large number of tumours. Researchers suggested that, you know, between a third and a half reduction in deaths from breast cancer due to this hormonal targeted treatment that came about from basic research.

And then we jump forward to, I guess, the late 80s and the 90s where the understanding of Dennis Slamon and the group he was working with of the impact of blocking growth factor receptors likes HER-2. And again, finding the right group of patients, using pathology to identify those patients by testing their tissues and giving them a targeted agent again as result in a huge improvement in outcome for breast cancer. So all those steps come together.

And what it means now is that, you know, in the current day, if you're unlucky enough to be diagnosed with breast cancer and I should say sites in the Western world and Australia in New Zealand, you've got a nearly 90 percent or just over 90 percent chance of still being alive five years later.

And that's really a remarkable change from, say, 20 to 30 years ago, where only 60 to 70 percent of patients would still be alive five years later from breast cancer. But obviously, there's still a long way to go, I'm sure.

I certainly know myself, you know, family, friends, you know, in your extended group personally who've been affected by breast cancer and while unwell, some haven't. And, you know, there's clearly a need for further understanding in those tumours that lack those specific pathways. That's we can target. We need to understand how we can treat those. So that's a real focus of ongoing research, obviously, you know, for the whole breast cancer community.

[Dr Michael Dray] So, Sandra, you've just covered 130 plus years of research in breast cancer. If I get you that we started in the 1890s and you've just described the massive improvement and the large and most common types of breast cancer, the massive improvement and survival outcome from that basic research.

That's a that's an impressive story. So coming back now, we're in the 2020s and we've got this smaller group of breast cancers that we're calling the rare breast cancers. You mentioned a phrase triple negative. Can you tell me more about that? And where research is going down those paths?

[Prof Sandra O'Toole] Yeah. So, look, as I was alluding to, is giving that background story that breast cancer is really divided up into what they express in terms of receptors that then decides often what their prognosis is and particularly what sort of treatment, if they require further treatment that they should receive. And so hormone receptors, the estrogen receptor and progesterone receptor, that sort of, you know, normally produced by the ovary, that the change with, you know, ovulation and pregnancy and menopause and all the different fluctuating levels in the impact that has.

Well, you know, in the order of 80 to 90 percent of breast cancers will express the estrogen receptor. And that is actually really great, because one of the most effective treatments are these hormone targeting treatments like Tamoxifen. And, you know, more advance treatments later on that are a bit more specific, and then probably about 10 to 15 per cent of breast cancers. And there's an overlap and will overexpress the HER-2 growth factor receptor. And there's a very effective treatment in that Herceptin trastuzumab and related therapies. And again, there's a whole new therapies coming through.

They're also looking even more effective. But that does leave a group of about, say, 10 percent of breast cancers that do not express either estrogen, progesterone or the HER-2 receptors. And they're called triple negative because they're negative for those three receptors.

And that's difficult for those patients, because the most effective treatments in breast cancer are those ones that are targeted. What's then left is chemotherapy, which although very effective, and it's been really important in improving outcomes from breast cancer, because it's not targeted specifically to the tumour cell in terms of what those receptors are being expressed. It can be less effective and can certainly be less toxic.

And so there's this group of patients who don't have access to these smart, targeted treatments. And therefore, it's a real gap in our knowledge. And we need to really understand better ways to diagnose what's being expressed on those tumours and therefore determine what treatments they ideally should be receiving. What we've really exciting new phase of development understanding and again, going back to the basic science. So all those years, people have been trying to understand and study the immune system, which is incredibly complex and difficult to comprehend.

And I find very complex. I think they don't name their things very well, all the CDs and interleukins. I think that it'll be more interesting names than that, because it I don't I find it less engaging then a gene called Sonic Hedgehog, for example. But the immunology research has for years for plugging away, trying to understand how the immune system worked and how the immune system wiped out, I guess, a lot of cancers before they ever actually developed. But then what happens at some cancers go on and develop and in fact, progress and escape the immune system.

So, again, years and years of basic research before, you know, research is really understood. And they won the Nobel Prize for understanding the so-called immune checkpoint. And basically, it's a way to keep various processes. But, for example, in cancer, I guess, basically it's a way that the cancer cells can evade the immune system, basically turn off the effective T cells that would come in and normally knock them out and allow them to evade the body's defences in terms of the immune system. So that's just this whole new era of immune checkpoint inhibitors. And the results have been incredibly exciting.

So breast cancer was in the first phase of these at all. It's been melanoma and lung cancer and a whole series of other cancers that have had really fantastic results with these. But again, although immune checkpoint inhibitors so far haven't looked very promising in hormone receptor positive cancers, it turns out the triple negative cancer is actually quite frequently have a very florid immune response associated with them.

So I'm sure, Michael, you've looked down the microscope to a triple negative breast cancers and they often have piles and piles of lymphocytes around the cancer cells, and some of those patients actually have really good outcomes despite the fact they've got a nasty triple negative breast cancer. The other cases, despite the dense immune response, it looks like it's doing something. It's actually not very effective. So recent trials have shown that patients with triple negative type of breast cancer, a proportion of those patients can respond to immune checkpoint inhibitor therapy.

And there's a whole pile of studies going on there with early testing just rolling out and some treatments becoming gradually available for those patients. But that's still very early phase research. And we've got a lot more refinement to do to understand which patients can respond to this treatment, at what stage they have the treatment, should they have it early? At the minute, it's really only available for patients who've got metastatic disease. But, you know, obviously the goal is to try and intervene before patients develop the advanced disease and actually improve their outcome. So I think a lot is happening and very exciting times in a whole bunch of tumours, but particularly in breast cancer, where it's got such a history of leading the way in understanding the biology and then translating that through into effective tests and treatments.

[Dr Michael Dray] A lot of this relies on tissue to do your research. Now, I understand you have some involvement with a with a tissue bank. Can you explain what is a tissue bank and how that is helping research?

[Prof Sandra O'Toole] Absolutely. So we're talking about basic research. You go back and you look at bits of cells that are growing in test tubes and in flasks and even mouse models. And those models and those cells that come from human patients years and years ago, growing in glass tubes are incredibly useful, an important tool to try understand the basic

processes that drive cancers, that may stop cancers growing and potential treatments that they really have their limitations because they're highly artificial. Obviously, mice and humans, although we share a lot of the biological processes, they're incredibly important differences. And the cells in the test tube is actually missing out on all the surrounding structures that are so important in supporting or inhibiting cancers.

So a lot of earlier research has really concentrated on cell lines. And the same sort of cell lines that are being used for decades and on these limited mouse models, but really in order to move things into practice is such a jump between the laboratory and actual live patients that we've got to work out how to bridge that gap. An incredibly important way to bridge that gap is by having the actual tumour tissues available to study. And patients are incredibly generous. And we've ended up setting up several tumour banks at different institutions over the years.

And one of the more recent ones that I was involved in setting up was the Sydney Breast Cancer Foundation Tumour Bank, which is based at Royal Prince Alfred Hospital. And there is a fantastic organisation. That's the Breast Cancer Foundation that was started by, you know, friends and families and clinicians at Royal Prince Alfred Hospital to put together the available for resources for patient care, but also very importantly, to support research. So when I started at Royal Prince Alfred Hospital, it was back in about 2009, 2010, we had this idea that we wanted to put together a large bank of tumours from which we could collect all the clinical information to understand which women had responded to the treatments, which unfortunately hadn't done well. And the second we study those tissues and use that as a bridge between the basic science laboratory and then hopefully on to a test or a clinical trial one day that we could then use to improve diagnosis and outcomes in breast cancer.

So Sydney Breast Cancer Foundation were really generous, and they gave us the money to support a research nurse and a scientist who helped us put together these large collections of tissue bank and most importantly, the patients who generously donated their tissues and agreed to it. So we saw that with sort of routine tissues that you have left over when you have your breast cancer surgery. And then it's examined by the pathology laboratory and the tissue blocks are then put into storage within wax.

And they can be useful for years and years and years. And they're there in case the patient needs any further tests done in the future on them and for various other sort of quality purposes. But we were able to use some of those tissues to put together collections of patients with specific types of tumours, whether it was

the triple negative cancers or the HER-2 positive cancers. And then more recently, we've moved on to patients generously giving us a bit of the fresh tissue that happens at the time of surgery and again, the surgeons have been fantastic, letting us sort of intercept tissue on the way to the path lab, we take it there while it's still fresh and take out some slivers of tissue that we can then use to do even more advanced studies on. We can grow those tissues in mice in a dynamic way to sort of see what drugs or genes can change in terms of treatment within those models.

And more recently, we've got incredible new genomic capacity to dissect out these tissues to the single cell level to understand exactly what's going on in thousands of genes, individual cells within the tumour. And that's particularly important for understanding the immune response to cancer.

And so my colleague, Alex Swarbrick, who's a professor at the Garvan Institute, has really been leading that research and working again with multi-discipline teams, colleagues here at Royal Prince Alfred Hospital at Concord Hospital, various other institutions, Lifehouse, to collect those tissues and follow up the patients.

And we really now have this living resource that we can use to understand in real patient samples how cancers might respond to changes in the immune system or changes in the expression of various genes or in response to drugs. And it's proving to be a very, very powerful technique that we're really hoping will let us understand why some patients develop metastases and why those patients metastases ultimately die through an autopsy series as well, with patients who develop metastatic disease incredibly generously and bravely have donated their tissues immediately after their death - for us to understand better the processes involved in that and then hopefully to change that in the future.

[Dr Michael Dray] It becomes a very powerful tool, doesn't it, for research and very much a blue sky thing. You're preserving stuff for the future, that and tests and technologies that you don't know what's around the corner next.

[Prof Sandra O'Toole] Well, absolutely. And look, I guess what really is amazing about this is that it has been a long-term project, and then it's often very hard to fund because research organisations often need to want to see results earlier.

Their funders, their donors want to see things are changing, things are coming out. And look, the tumour bank, again, that's been a long term process. It started off with paraffin tissues back in 2010. I'd say about the past three to four years, we've really moved into fresh tissue collection.

And it's incredibly powerful to, you know, collection, but it's taken a lot of time and money and characterisation to bring it to this point. So, you know, we're really grateful for the funding and the generosity of patients, you know, involved in that process in the time of all the clinicians as well, who don't obviously don't necessarily benefit directly from having their surgery slowed down. You know, at the end, waiting a bit extra time for us to take that tissue. So, yeah, it's a really it's a something that people do with an eye on the future in the eye for the greater good.

So, you know, it's really great working with people like that.

[Dr Michael Dray] Yeah, and I certainly agree. Now, the Australian National Breast Cancer Foundation has an aim to reach zero deaths from breast cancer by 2030. How are you involved with that process?

[Prof Sandra O'Toole] Yeah, so look, the National Breast Cancer Foundation, it's a really great organisation. I mean, I have to say, has benefited from direct funding from them. But I'd say for at least six, maybe seven years, I've served on the research advisory committee, and that's a group of interested and expert individuals in a variety of fields, you know, all related to research, whether it's more clinical end or more of the basic science and or more in the nursing end, whichever way you're looking. So they've got quite a broad expertise with that. They're really very focused on what they do and their goal, as you've just stated, zero deaths by 2030.

Now, that's a big that's a big goal, a really ambitious goal. But I think really without those goals, sometimes you get lost on the long journey. Some of these projects can take a long time to come to fruition.

So what the National Breast Cancer Foundation have been doing is trying to understand directly what sort of projects and what sort of research and strategies can they be supporting to that directly lead to that goal of zero deaths.

And, you know, they've been really strategic about it. They're funding the early science projects that we hear are important, but also a focus on areas of need. So triple negative breast cancer, metastatic cancer, areas of need and understanding areas of inequity as well.

I mean, if you look at, you know, outcomes in different communities, obviously they're not as good as in, you know, better off, better served communities. So although they cover the full gamut of research and implementation, I think they're very strategic in how they're doing that.

And they've got a you know, as I said, a. Broad group of people advising them on the best ways to do that and they're a lean organisation, they're really focused on what they do. The money that goes to them is going into the research, keeping all the other costs down to a minimum.

And so, yeah, I'm really impressed with what they're doing. It is a really tough time to be involved in research in terms of funding without funding. You just actually can't do any experiments or move things forward. And we're actually losing a lot of young scientists out of this process because the process of funding is so competitive that very good research will just not be funded because there is not enough money to go around. So some of the research success rates are getting down towards 10 percent. And if you spend, you know, two to three months of your year as a productive researcher, writing grants that ultimately don't get funded, it's a pretty disheartening process.

And ultimately, you just simply can't keep going. You can't even cover your salary. So I think having organisations like that that are more nimble than some of the larger funding organizations have very, very focused goals, can really have some great successes.

And they've really linked with their community as well. So they do a lot of community engagement. And consumers are directly involved in the research advisory committee as well, to sort of say what they as consumers, patients who've had breast cancer, what they want to see come out of this funding that's come from the community.

And they were really leading the way in that sort of consumer engagement that I think is incredibly important.

[Dr Michael Dray ] We were talking earlier about your passion for new things and new technologies and different ways of doing things. And perhaps we can segue off that to an article that I've got on my desk here, and I'll read the name out. It's Colorimetric histology using plasmonically active microscope slides. And tell me a bit more about this project?

[Prof Sandra O'Toole] Ok full disclosure. I am not a physicist. This is where it comes about working with really smart people. And that's one of the things I really love about research, is how you bring people together, different backgrounds, and you can sometimes address some really difficult questions that otherwise would not be addressed.

So way back in the 2000s, I think what it was must be about 2003, 2004, I was working at Johns Hopkins University in the States, and I met this lovely Australian woman who was doing her postdoctoral studies over there as a scientist in metastatic breast cancer. And our lab heads said, oh, you're an Aussie, she's an Aussie. You guys should have a chat to each other. And that's Belinda Parker, who's a scientist at Peter MacCallum Cancer Centre. Anyway, Belinda and I, as well as working together, became very friendly and stayed in contact over the years and then started doing research together. And, you know, she's had a lot of success and a lot of interest in immune responses in cancer and also in mouse models. So we're doing a lot of work together on characterising these models of mouse mice, developing tumours that could be used as models to mimic the human process.

Belinda spent some time at La Trobe University where she was chatting with some physicists. And these physicists would normally not have anything to do with people in the biological clinical fields at all. But through those discussions, these guys, Brian Abby and Eugeniu had developed these microscope slides that have got this tiny sub-microscopic grid on it with tiny little holes in this sort of a nanoscale on them. And what that does is it when light passes through that like it normally does, when you put a glass slide on a standard microscope and the light shines from below to illuminate the tissues.

When you shine that light through in that tiny nanoscale grid is on the microscope, it actually changes how the light refracts and how the light behaves. Now, Michael, as you and I both know that when you take tissues from a human body and we process them like we normally do to make microscope slides, when you cut those sections

very finely to put on a glass slide, they pretty much see through all like a white sort of colour. And if you look down the microscope without any extra chemicals added, you can't see very much at all. So what we do is, you know, as pathologists, that we take a series of dyes that most of which came from clothing dyes, from, you know, 100 or 200 years ago. And they're taken up differently by different parts of the cells. And we then able to look down the microscope and based on the different colours, we can start to interpret what tissue we're looking at, whether there's cancer there and all the important stuff we need to know in determining patient diagnosis and therefore subsequent treatment and outcomes. So what these guys realise, these physicists realise, is that if they put different tissues on this glass grid, that in fact different parts of the tissue change colour without any stains being ever on top at all.

So they don't have to do any further processing. You just cut the section, put it. On the slide, and when you put the light through it, just like on an old microscope. Different parts of tissue are totally different colours based on various things, including density.

So these physicists were chatting with my colleague Belinda, and she then said, well, obviously the uses for this are huge we need to be looking at these mouse models to prove whether to understand whether, you know, cancers look different under this process and because Belinda and I been working together for so long. She then contacted me asking if I could be involved in assessing the tissues using this new microscope slide. So that's, in fact, what happened. The physicists have done their amazing, clever stuff and invented these nanoscale grids. They call them nano slides.

And then Belinda and I were then deeply involved in the process of trying to translate this into something that can be useful clinically. So I should say this paper is really only the first sort of proof of concept idea that we've taken a mouse model of cancer. And we realised looking down the microscope that as the model had developed more and more advanced lesions, looking, you know, from precancer through to invasive cancer, there was some quite significant changes in colour in the cells without adding any extra chemicals.

And then we also looked at some human tissues. And one of the challenging areas is, you know, Michael, in breast cancer is like when you cross the line from a lesion that is just proliferating to one that is actually cancer because the treatment and outcome is very different for patients at that point.

And it can be very difficult even for experts to understand when that line has been crossed and when a patient may just be observed closely or when they may need a mastectomy, for example. So it's a really high stakes decision.

So in this early proof of concept paper, we've certainly seen that it looks really promising. It looks like the cancer cells have very different properties when put on top of this grid and they really stand out. And because you don't have to do any further processing or chemicals, it's going to be a very quick process.

So as you know, you know, when you sit around waiting for the extra tests that we do, the chemistry on our tissue, that can add days or sometimes even a week or more to the process. And if patients are needing a rapid diagnosis and therefore ongoing treatment, that can be a really serious delay.

So we're really hopeful that with further studies, this technology may prove to be really, really useful addition to our tools as a pathologist that will let us make a quicker and more accurate diagnosis in a whole range of areas. Obviously, we started off in breast cancer because that's our interest. But I think there's many, many other areas that we can be looking to see. Can we get a better diagnosis using this this technology? And at the minute, because it's custom made, it's a bit expensive.

But looking into mass-producing, this, you know, I think it could be highly affordable technology that can be used, you know, even in countries that aren't, you know, haven't got a really advanced health system. So we're pretty excited about it. And I think it shows the power of, you know, different groups from wildly different backgrounds coming together with a mutual passion to sort of find out new things and improve the way we do things.

[Dr Michael Dray] I think I agree with everything you said. I think this is a fascinating paper and that it's talking about a replacement of our histology bread and butter: hematoxylin and eosin stain which is a 19th century industrial dye with a 21st century nano textured piece of glass. And I think that's just awesome. What can be achieved with this? And I agree. I look at the model of radiology, where they've gone completely digital and they've missed out the development of photographic plates and they've gone straight to digital to streamline and bring the histology laboratory into the 21st century.

We're looking for something that that actually we can replace that big chunk of the laboratory process with. And something like this may revolutionise in years to come, decades to come. But the histology laboratory and bring us into the 21st century from the 19th century. So I think this is just fascinating creativity of people to do that.

[Prof Sandra O'Toole] Yeah I know it is I mean, I should say, Michael, that like, you know, it seems what we do is pretty retro. You know, using sort of microscopes and slides and these dyes from, you know, 100, 200 years ago. But I guess the more I observe, you know, tumours down the microscope and then we do molecular testing and understand the genetic processes that although it seems a bit retro, in fact, looking down the microscope in more cases, we understand more actually gives us a really fantastic snapshot understanding of all the processes that have occurred to bring us to the point of this patient having a tumour.

We can look down the microscope, we can see the immune system. We can see the microenvironment. We can understand. And at what stage the tumour is, has it gone to lymph nodes, has it invaded blood vessels? Does the patient need more surgery? Has it gone to the margin? So although it does seem retro, I think what we need to supply our knowledge to understand what resulted in that appearance, because ultimately that is

just it's really quick snapshot of all these processes that have gone on before it to reach this point.

So I'm hoping, at least for now, this technology might help us do that better. But I still see a very long role for the standard microscope, slide and histopathology.

[Dr Michael Dray] I agree on that. And often with understanding through new technologies and tests, we're able to reverse engineer that understanding to what it should look like on an H&E stain. And we can make diagnoses very, very effectively and problem solve very effectively just on those H&E slides. And we use them, as you say, to predict the future. We're predicting how this tumour is going to behave. And it's going to take a lot to replace that.

[Prof Sandra O'Toole] No, absolutely. But, you know, I see us as pathologists, really as a key member of the team, and I see us particularly in that that space between the laboratory, the basic science laboratory or even the translational laboratory and the patient.

And I think we've got really unique skills and experiences that make us a really important member of those teams that, you know, sometimes we, you know, I guess not recognised or appreciated as perhaps we might be. But I think that's changing as time goes on. And thanks to, you know, initiatives like this with the podcast as well.

[Dr Michael Dray] Sandra, thank you very much. It's been a great pleasure talking with you this afternoon.

[Prof Sandra O'Toole] Oh, thanks, Michael and as always, a pleasure to talk to you as well.

[Voiceover] You have been listening to The Pathologist Cut podcast with RCPA president Dr Michael Dray. For the latest RCPA updates, make sure you're following us on Facebook and Twitter.