NETWise
A Podcast About Neuroendocrine Cancer

Episode 19
NET Research-Part One:
Transcript

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Welcome to NETWise. This is a podcast for neuroendocrine cancer patients and caregivers that presents expert information and patient perspectives. I'm Elyse Gellerman from the Neuroendocrine Tumor Research Foundation.

Today is the first of two episodes on a topic near and dear to us here at NETRF: cancer research. Advancing NET research is the core of our mission, and it's a crucial need, because NETs are extremely complicated cancers and there is still a lot the scientific and medical communities need to learn about them.

Here's Dr. John Kanki, NETRF's Director of Research, followed by Dr. Chrissie Thirlwell, Professor of Cancer Genomics at

University of Exeter in England and Co-Chair of NETRF's Board of Scientific Advisors:

> ***Kanki:*** *"As we've learned more and more about NETs and the biology that underlies it, that biology is becoming much more complex than originally thought. People thought, 'Well, we'll find a gene that is involved with NETs. We'll figure out a way to address the treatment of that genetic problem and be done, and it's so far away from that and so much more complex."*

> ***Thirlwell:*** *"These really are unique and very, very different cancers to any other cancer. And I often call them intriguing, but I could easily flip that and call them absolutely infuriating because they don't follow the rules of lots of the other cancers that I know about and that I've treated and studied over the years. So they have a very different, very, very different biology and makeup to other, more common cancers."*

We've spoken in a previous episode about how clinical trials are conducted, but a clinical trial is just the tip of the iceberg: it's the visible end result of a tremendously long and complex process that takes a promising scientific idea and turns it into a usable treatment.

> ***Thirlwell:*** *"When we think about setting out in research to try and end up with a medicine at the other end of the line, there are many, many steps, and that whole process*

from identifying a target in your first experiments in the lab to actually drugging and giving a new treatment can take up to 10 years or so."

Here's Dr. Dawn Quelle, a professor of Pharmacology and Neurology at the University of Iowa, and also Co-Chair of NETRF's Board of Scientific Advisors:

Quelle: *"And in fact, that could be quite quick. We're often looking at more than 10 years. It takes a while to conduct the investigations, and then you realize, well, that raises five other questions, and maybe this result isn't that meaningful unless we show X, Y, and Z also happen. And that's the way research is. Often you can choose the wrong track and you don't realize it until you've gone a few years down the road.*

And scientists feel like we're working in the laboratory to improve patient health, but it can seem like a long road on that journey, and in many cases, it really is. It's not easy to take your findings and get them into clinical trials. I mean, you need hundreds of thousands of dollars just to do a very small clinical trial. So there is a pipeline that people need to go through. And I think it needs to be that way because we need rigorous science to actually guide and justify what we are going to do in a living human being."

> ***Kanki:*** *"So, it's quite long, and the amount of research that it takes to lead up to that will involve many, many dead ends. Nonetheless, we have to pursue them if we're going to find the answer."*

This process is the subject of today's episode. How do scientists do research, from beginning to end? And what are some of the challenges that specifically face NET researchers as they work through that process?

In science, everything starts with a question. "How does that work?", "What would happen if we did this instead of that?". Choosing the right question to ask is the first and probably most important step in the process.

Here's Dr. Ramesh Shivdasani, an Oncologist at the Dana Farber Cancer Institute at Harvard University and member of our Board of Scientific Advisors:

> ***Shivdasani:*** *"Really everything is driven by the question. You might look at a colon cancer and say, "How is this different from the normal colon?" And therefore, how I might define treatments or approaches that would push the cell either back to its normal physiologic state or how my treatment might kill the cancer cells without permanently damaging the normal tissue. And so that would be an example of a question. And from exposure to the field, from reading, from thinking, from assimilating, you understand*

where the gaps are. You understand where the gap is either conceptually or factually, and you frame a question that is answerable with experimentation."

Quelle: *"How do you make sure that your idea is testable? First, it has to be a rational idea and through discussion or thinking about it with others, you can figure out, 'Well, that's a great idea, but it's totally impractical. We can't do that" or, 'That's not really a good idea.' And a lot of times we have ideas and then we realize, 'No, that's really not good.'*

You get ideas through analysis of what is already known, so reading the literature, talking with your colleagues, attending scientific seminars or scientific conferences. You look at what you're already doing. And a lot of us think about, "What can I do in the context of what I know in this particular area?"

The nature of cancer is that it is a disease of uncontrolled growth. All the cells of your body have the ability to grow new cells by splitting themselves in two. This is a normal part of maintaining your healthy body. But cells built to be part of the function of our liver, or pancreas, or bowels, or lungs, or wherever — sometimes lose control and divide in a way that is out of balance with the cells around them. This is what creates tumors. Cancer is a dangerous persistent tumor growth.

Kanki: *"If you think about it, what cancer is, are cells that are dividing out of control and they're spreading around and causing havoc on the rest of the function of the body, and understanding how that process stops normally is*

in many ways what our therapeutics for cancer are involved with now, which is how do we stop proliferation and what's going wrong in the normal process of development that causes these cancer cells to just continue to proliferate unchecked? And the kinds of things that went on or went wrong when diseases such as cancer form actually represent biological pathways normally used during normal development, but then go awry."

Shivdasani: *"In other words, a normal kidney cell or a liver cell behaves normally, does its physiologic functions and goes on with life. A cancer cell is the very same cell that is now breaking the rules. It's either proliferating, replicating, dividing more than it should, or it is moving from one place to some place it doesn't belong and it is violating the rules of that organ system or that tissue. So, one very central question in cancer is how does a cancer cell anywhere in the body, how does that cancer cell differ from its normal counterparts?"*

These kinds of really fundamental questions — why do tumors start growing? What are the mechanisms that should be keeping them in check, and why are they failing? How is a cancer cell different from the healthy cells that surround it? — are great

examples of what is called "basic research". This is the kind of research that might seem the farthest from a working clinical trial, but without finding answers to these kinds of questions, nothing else can follow. This is the foundation for all new ideas in cancer treatment.

We've known for a long time that cancer is closely related to genetics. Something inside the cell's DNA or RNA is causing it to go rogue and form a tumor. And so, since the sequencing of the human genome began in the early nineteen nineties, much of the important basic research in cancer has had a genetic focus.

> ***Kanki:*** *"DNA, which is the building of all living organisms, contains what's called a genetic code, and the code is a very simple set of chemicals in a certain sequence. The sequence, though, is thousands and thousands and thousands... It's basically huge strands of nucleic acids, the sequence of which is incredibly complex. There's certain genes that seem to be mutated a large percentage of the time in a particular type of NET. Because of that prevalence, it's pretty clear that that mutation does something that predisposes people to get that type of NET."*

This makes it sound really simple: find the right gene, knock out the cancer. Unfortunately, it's way more complicated than that. Our genetic code is made up of combinations of only four proteins — represented by the letters A, C, G, and T — but there are THREE BILLION pairs of these letters in the nucleus of every

cell, and there might just be one faulty gene. It's the ultimate needle in a haystack.

> ***Thirlwell:*** *"It depends which of those three billion letters have changed. You can literally have a one letter change, which stops a whole gene from working properly and protein working properly"*

It's even MORE complicated than that, though, because genetic mutations can affect one gene or several, so the difference you're looking for might be in two or three base pairs that are billions of pairs apart from each other.

> ***Kanki:*** *"Or, it may be a combination of them, multiple genes acting together."*
> ***Thirlwell:*** *"You could have 50 different changes, so it's all about the position of where those changes are."*

The possibilities are literally almost endless. And as if that weren't complicated enough, when you do find a genetic abnormality in a tumor it's very hard to tell cause from effect, whether that change is the cause of the tumor or the result of it.

> ***Kanki:*** *"Now you can imagine that the list of this could be hundreds of genes and pathways that are affected. And so then you have to figure out which of those genes and pathways are really relevant to the disease, which ones are responsible for causing the disease and which ones are just*

because the disease forms, are a consequence of the disease. That gene may be involved in many different processes, and we don't know which of those processes yet is really the key one."

A piece of good news is that there has never been better technology than there is right now for analyzing massive amounts of data.

Thirlwell: *"The rate and pace of how we analyze the data has shot off exponentially. The technology is absolutely amazing. The volume of data we create now is incredible. We work with data scientists and bioinformaticians who are absolutely brilliant at handling this massive volume of data and working out what's significant, what's insignificant, if this change in the DNA sequence is going to really affect the protein so that we can actually use computers just to work and model this really quickly to look for the downstream effect. So you can literally type in the mutation that you found and you can get an artificial intelligence, or you can have a data science model that tells you what the downstream effect of that is. So that's the rate and pace of the technology and the bioinformatics around this as well."*

There's also recently been game-changing technological developments in how to *manipulate* DNA.

> ***Thirlwell:*** *"You can edit DNA. So there's CRISPR editing, where you could actually select... once you've identified your target, you can use CRISPR editing. It won a Nobel prize recently, CRISPR editing to identify where your sort of area of interest is, and then you can change that and modify it. Prior to that, we had other ways of... we used to use what was called a lentiviral. We used to use a viral approach to go in and modify and change genes, but CRISPR is the most commonly used approach globally now."*

So hard as it sounds, real progress has been made in the genetics of cancer.

> ***Thirlwell:*** *"In the whole of cancer research, the sequencing has been really, really helpful in speeding up that whole process of identifying a target and then giving patients a drug and a new treatment."*

Unfortunately, NETs have been much slower than other cancers to yield their secrets to these new techniques.

> ***Thirlwell:*** *"In neuroendocrine tumors, it's much more challenging, because with all of the sequencing studies that have been done so far we don't find these actionable mutations."*

This challenge has to do with the unique biological characteristics of NETs. To start with there are very few

healthy NET cells to compare to the cancerous ones, and they are extremely difficult to find and analyze.

Shivdasani: *"In the case of neuroendocrine tumors, we immediately reach an impasse, and the field has been at that impasse for decades now. And that is unlike, say, colon cancer or stomach cancer, where the cancer is a disease of the bulk population in that tissue, what we call the epithelium. In the case of neuroendocrine tumors, the cancer has affected a cell that ordinarily occupies less than 1% or less than 0.1% of that tissue. And so if you*

simply want to compare the cancer to the tissue, right off the bat you're comparing apples to oranges. In the case of your ordinary epithelial cancer, like colon cancer or stomach cancer, you're comparing Gala apples with Fuji apples, but in the case of neuroendocrine tumors, you're comparing a tumor which is made up of billions of cells of a particular type, but that cell type is so massively underrepresented in the normal tissue that if you compare the two tissues at face value, you will simply extract nonsensical information.

If I were to compare your kidney with your liver, just grind it up and take a look, I would see thousands of genes that distinguish those two organs. Similarly, I can take a normal colon tissue and a normal colon and a run of the mill colon cancer and I would find hundreds of differences that could get me thinking about how to tackle the problem

in the clinic. But if I'm doing that with neuroendocrine tumors, I don't have a comparator. I don't have a reference that has any meaning because the cells that are pertinent are so sparse.

So people have tried many different approaches, all conceptually sound, but have consistently hit a brick wall because that paucity of cells is so extreme that even if you enrich tenfold, twentyfold, fiftyfold, you're still operating with a profound limitation."

Despite these limitations, though, NET researchers around the world do find actionable, testable basic research questions, some of which are then brought to the next stage, which is called "translational research."

Kanki: *"'Translational' means whether a finding in a simple biological system will translate into humans, moving the ideas and therapeutic strategies that are developed in non-human work and then testing them in models that, if they're successful, may justify the next step, which is trying them out in actual humans and in clinical trials."*

The idea here is moving an idea — translating it — from the language of biochemistry into the language of medicine.

Quelle: *"So there's a continuum, I think, of basic research and translational research, and they kind of intersect in*

the middle at the far end of really basic research. That's where people are doing studies of basic processes in a cell that could be relevant to anything. Maybe just how a normal cell survives. How does, maybe, protein synthesis occur within a cell? How does DNA get replicated in a cell? Very basic question."

Thirlwell: *"And then we can identify changes that we think might be important in terms of looking after patients in the clinic and I feel that's where the translational research starts. And then we can take it further forward."*

The main mechanism of translational research (and much basic research as well) is creating "models" — ways to approximate the behavior of human cancer cells so you can test ideas for possible treatments without having to test them on actual patients. One of the ways to do this involves what are called "cell lines".

Thirlwell: *"So, cell lines are where you have some cancer cells and that you are growing them literally in a flask. You keep them warm, you give them food, you put them in a nice, warm place to grow."*

Quelle: *"These models are taken initially from a patient tumor. It is brought into the tissue culture room and you mince up the tumor and you treat it with enzymes that will*

dissociate all the cells. Then what you do is you can put those cells just into a dish, and if they form either adherence cells on the dish or they can grow in suspension, and if they are able to continue growing under those conditions, then you may have a cell line."

Thirlwell: *"And then in that cell line, you can knock out the target that you're interested in and see what those cells do. They might die. They might grow faster."*

Kanki: *"Now, there's a lot you can do with those kinds of tumor cell lines, and we can test scads of different drugs and things on whether or not they'll kill those cells or not."*

An interesting thing about cell lines is that they really only work because the cells involved are cancerous, and therefore growing at an abnormally fast rate.

Quelle: *"Normal cells, if you were to take them out of a tissue and put them in a dish, they can only grow and divide maybe 10 or 20 times, and then they die. But tumor cells have an advantage because they're already transformed, and so they have an advantage and ability hopefully to be immortal and to continue growing in culture."*

When this works well, these cell lines are called "immortal", meaning they can be kept alive indefinitely, and also divided up

into multiple cell lines, for use by researchers around the world. But the propagation of cell lines is more difficult in NETs than in many other kinds of cancer. Because the most common forms of neuroendocrine tumors grow very slowly, they are closer to the behavior of normal cells than more aggressive cancers, and therefore it's very hard to turn them into viable cell lines.

Thirlwell: *"It's quite easy to create cell lines in lung cancer, because they grow really quite quickly."*

Quelle: *"With the neuroendocrine tumor cell lines, it's just been a huge challenge. People have been trying this. My lab tried it for several years. We thought we had a few*

going, but after four to five months, they kind of stopped growing on us and we lost them."

Thirlwell: *"You can't actually then work out or study the biology to see if your target is making things grow faster or slower because they sit there for three weeks and then they die. And I think that just reflects the indolent and quite slow growing nature of some of the neuroendocrine tumors."*

Quelle: *"And they seem to have very particular requirements to get them going. So I think there has been a lot of effort by those in the field. I think we're starting to overcome it, but we're not there yet."*

While there are hundreds of viable cell lines currently being used for other kinds of cancer, for neuroendocrine tumors there are currently only three. All three were developed from Pancreatic NETs, and therefore only represent one segment of the NET community.

> ***Quelle:*** *"We have two cell lines that have been around for thirty years. They represent non-functional pancreatic NETs, which are the majority of the tumors seen in patients, but what people don't like about them is they grow so rapidly — more Grade-3-like — which is not like the typical tumor cell from the patient.*
>
> *Most recently, in the past three years, we had a third cell line that was added. The newest line that was developed reflects an insulinoma cell line, so this a functional pancreatic NET cell — not the most common type of tumor seen in patients. I hope we will develop more sustainable cell lines."*

While the quest for more and better NET cell lines continues, another technology has been used to generate NET cells for study. These are different kinds of human cell cultures called "organoids" and "spheroids".

> ***Quelle:*** *"Organoids and spheroids represent another opportunity in the field and they… we have seen really wonderful developments."*

Unlike in cell cultures, these are portions of human tumors that are removed and grown as miniature blocks of tissue, preserving both cancer cells and some of the other kinds of cells that surround them, mimicking the biological environment where they came from.

> ***Quelle:*** *"The big advantage with these is that they come directly out of a patient. How we define them I think might vary between one scientist to the next. I would say that a spheroid is perhaps less… has a lower number of mixed cell types within it. It's more homogeneous. Whereas an organoid has a greater variety of cell types that are adding in to*
>
> *it, and perhaps may reflect the actual tumor a little bit better."*

While organoids and spheroids are not immortal like cell lines, they can be kept alive long enough to do some interesting research. They're also derived from a particular tumor in a particular patient, so they may potentially be used for personalized medicine — figuring out which treatment is likely to work best against that specific tumor.

The challenge with them is that they can't be kept alive as long as cell lines, and they can't be coaxed into reproducing, so while a cell line might generate strains of cancer cells that researchers use for decades, an organoid or spheroid is only useful in that particular lab and only for a short time.

Quelle: *"The sustainability of those spheroids and organoids is the issue. We can't propagate them. We can generate them, but they often just sit there and they can be viable for nine months, but they aren't growing and they aren't able to be propagated so we could take one well and make it into 10 wells and so on. So we have a limited window of opportunity to do analyses on the spheroids and organoids right now. And I think there's a lot of effort across the globe trying to figure out 'what do we give these cells to make them grow better so that we can propagate them more effectively?'"*

Thirlwell: *"I really feel we're finally making some progress in terms of some of the cell lines we use and the organoids that have been produced, so I think that's fantastic. There's still further work to be done with those organoids to either make them more reliable, and we're getting to a point where they are being used by other groups and they might be manipulated. So there's quite exciting data coming out of that."*

Another kind of model that is often used for experimental testing is animals, often mice or zebrafish.

In order to begin an animal trial, researchers have to go through an extensive review process to make sure that the

promise of the idea they want to test justifies the expense of caring for live animals for the months or years the experiment might take to run, and also that they have the facilities and expertise to humanely care for the animals. When these requirements are met, animal trials can tell us things about potential treatment options that test-tube experiments just can't match.

> ***Kanki:*** *"Take, for example, a mouse. And let's say we transplant a human NET tumor into it, and we can make it so that that tumor will grow. We can engineer the mouse so that it doesn't attack that tumor on its own by its own immune system and the tumor will grow. And then we can see whether or not we can inject that animal or treat it with certain drugs."*

> ***Thirlwell:*** *"You can turn genes on and off in mice, either at a very high level in terms of the genes that are in every single cell of the body, or you can use mechanisms to turn them off in the bowel or in the pancreas or places like that, to learn more about that biology."*

> ***Quelle:*** *"So that makes it a great model system that replicates the human disease in one context, and gives us a way to test our ideas."*

This stage is crucial, because even the best cell culture models only get us part of the way to understanding how cancers work and what techniques to fight them might be effective.

> ***Quelle:*** *"When you're working in a cell that's in a culture dish, it's a very atypical environment for that cell to be growing in. So that's one reason why sometimes there are studies that we're doing in a cell that may not actually translate to what is happening in an animal and you won't know it until you do it."*

And when it comes to safety, there's also really no way to know what negative effects a treatment might have on people without first testing it in animals.

> ***Quelle:*** *"And we don't want to hurt people, so we need to have as much justification as possible from pre-clinical work."*

And so these tests are extremely useful. They're not perfect, though, because mice aren't human, and their biology is not exactly the same.

> ***Quelle:*** *"Now, sometimes there's that issue of whether we can cure cancer in mice, but to make that leap into people, it's very difficult. Mice are different than people. They have different genetics. They have a different physiology, anatomy, everything. Their metabolism is different."*

Attempts are being made to find better animal models, ones that would respond more similarly to humans. This means using mammals whose anatomy is closer to ours.

> ***Quelle:*** *"That's why some people, and I even got involved in some projects like this, where we've worked with large animal models of particular tumors. So we've worked in mini- pigs. Genetically, anatomically, physiologically, they are so much more like humans that there is a benefit to doing that and there is a movement in cancer research to use more of these animal models."*

There are problems here, though. The ethical considerations are more complex, and keeping and caring for pigs is an order of magnitude more difficult and expensive than keeping and caring for mice. So this is still a developing area, and not nearly as common as working with smaller animals.

The next step, if something continues to work well in animal testing, is to move to clinical trials, where new treatments are tested in people. We spoke about the clinical trial process in-depth in Episode 7 of NETWise, and I encourage you to go back and listen to that podcast if you haven't already.

To sum up, clinical trials work in several stages, first testing a new treatment for safety, and then for effectiveness. It's a slow, methodical process and very, very expensive, so only treatments that have demonstrated real promise in those earlier pre-clinical stages are considered for clinical trials.

And unfortunately, there's a real tendency in the community, the media, and among policy makers to pay lots of attention to

developments that have reached the clinical trial stage, and not enough on the basic and translational research that precedes it. Clinical trials are just the last stage of this process, and they cannot happen without years of careful pre-clinical work, which is often underappreciated and underfunded.

> ***Shivdasani:*** *"You know, we are all probably wired for instant gratification, and I'm continually struck by even the most well-meaning and educated and sophisticated patients or donors. 'Tell me doctor, what's going to be a clinical trial next year or this year? What can I do to push that envelope?' With actually a very limited awareness that the vast majority of clinical trials fail, an overwhelming majority of clinical trials fail, and the reason they fail is not because the intentions were bad or the experiment was designed poorly, it's because the foundations in which they're based are spartan. If you don't really know what a problem consists of, you can only frame the question with what you know, and often these questions in the clinical trial setting are naive or simplistic because that foundation of basic understanding is limited. So, basic research acknowledges that you lay a foundation literally brick by brick, stone by stone, with the idea that the concepts that come out from that are fundamentally related to the root cause of a disease, and every major advance in medicine has come from that deep understanding."*

> ***Quelle:*** *"And you won't know it until you do it. There is really no good way to predict whether it's going to work, so you just have to do the experiment."*

And this is why NETRF has always made this basic research the core of what we support. Because this is where the next important treatment for NETs may come from.

It's not easy, though. You may have noticed a pattern in this episode. At every step of this process there is either something about the nature of NETs or the practical realities of funding science that makes it challenging to do basic and translational

research. The truth is that while there are more good laboratory models for NETs than there have ever been, there are far fewer than we need.

> ***Kanki:*** *"There've been a lack of models in NET research, and it serves as a bottleneck for being able to test which pathways and what genes and what processes are really relevant to NET formation. And without that, we can't then really take the next step, which is testing whether or not certain treatments that affect those pathways may work in humans or not."*

> ***Thirlwell:*** *"It's the models that have been missing. So you can identify your targets and you're missing that really*

vital step to then get to the bit where you might start being able to identify drugs for human use."

Despite these challenges, though, amazing work is being done on NETs right now, much of it with funding from the NET Research Foundation. So that's what we're going to explore in the next episode: what has been the history of NET research, what is some of the most exciting science being done right now — some of it by the scientists we met today and their colleagues — and what might the future of NET treatment look like?

Thanks for listening to NETWise. I'm Elyse Gellerman, CEO of the NET Research Foundation. This episode was written and produced by David Hoffman of CitizenRacecar; Post-Production by Garrett Tiedemann *(TEE-da-min)*; Production Manager, Gabriela Montequin *(mon-ta-KEEN)*. It was made possible by the generous support of Ipsen; Advanced Accelerator Applications, a Novartis Company; TerSera Therapeutics; and Progenics Pharmaceuticals, a Lantheus Company. Special thanks to everyone we interviewed for this episode. We are grateful for your expertise. This is a production of the Neuroendocrine Tumor Research Foundation. We're committed to improving the lives of patients, families, and caregivers affected by neuroendocrine cancer by funding research to discover cures and more effective treatments and providing information and educational resources. Please visit us at NETRF.org

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NET Research-Part One:
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