00:00 Introducing Thanos Tzounopoulos – Director, Pittsburgh Hearing Research Center – University of Pittsburgh

Hazel: Welcome to the **Tinnitus Talk Podcast.** I'm here with **Dr Thanos Tzounopoulos.** Welcome, Thanos.

Thanos: Thank you very much. I'm happy to be here.

Hazel: So, you may or may not know this but ever since we started this podcast, now over a year ago, we have gotten many, many requests to have you on, so it seems like you have a lot of fans out there!

Thanos: Happy to hear that and hopefully our discussions will be helpful to them and, as I said, I'm happy to be here.

Hazel: So, we live in strange times obviously so I feel like I would be remiss if I didn't start by asking you how are you coping under the current circumstances and how is your work affected?

Thanos: Thanks for asking. Things are fine overall. We are fine. My family, and my community, everybody seems to be doing fine. Of course, as you know we are all experiencing unprecedented times and we all have to adjust and regarding my work, the lab has been basically under partial lockdown. I will explain what that is, for the last three or four weeks because we have animals obviously in the lab, we are allowed to have essential personnel so we can go there and take care of the animals – mice in my case, for the most part. Then, a few experiments that had started before the lockdown we were able to complete and will continue them otherwise it would be to the detriment of the mice. However, we cannot start any new experiments and we basically operate under these essential personnel. So, no new stuff going on right now, but we understand that, and we are trying to do the best for ourselves and our community. How are things there? How is everybody there?

Hazel: Yes, so I'm recording from Finland. Things are I guess not as bad as in some places in the world and I know in the US and some places like New York City it's pretty bad, so I guess we're relatively ok here.

Thanos: Good, good to hear.

Hazel: So, you mentioned delays in the lab work. Are you worried not just about your own work but in general, delays in clinical research?

Thanos: Again, right. Sometimes priorities change depending on what life is bringing us so, sure, my work and other people's work is going to fall behind a little bit but it seems that it's necessary, what we are doing right now in order to avoid worse things from happening. So, although I cannot speak for others it seems that overall, at least so far, the measures seem well-justified and nothing horrible to my knowledge has happened because of the stoppage of this research. And, as I said, essential research is still going on. However, given now that things are going slightly better, at least they don't get as bad or they don't evolve as bad as they did a few days ago, there has been talk about repopulating the lab, you know, gradually, and getting back to some sort of work. But again, the specifics of how these things are going to happen, it's unclear yet given the uncertainty of what we are going through.

Hazel: Yes, let's hope so. So, let's talk a little bit about your work.

03:54 Background and current research

Hazel: You are the **Director of The Pittsburgh Hearing Research Center**. Can you tell us a bit about what it is you do and how it relates to tinnitus?

Thanos: Yes, maybe can I start a little bit earlier about how my journey is? It's individual. So basically born and raised in Athens, Greece, then I studied biology at college in Athens, Greece, general biology at the time and then the last couple of years I focussed more on molecular biology and then through a **Fullbright Fellowship** I came to the United States to do my PhD and I went to Portland, Oregon, where I studied biophysics of potassium channels. These are these membrane proteins that allow potassium to go in and out and are very important for firing the activity of neurons. We were trying to understand how these channels open and close and how they perform their function, and I did that under the supervision of **John Edelman**, who was my mentor. Then after that I went to **UCSF,** I went to **San Francisco,** where I did my post-doctorate with **Robert Malenka.** At the time **Rob Malenka** was running his lab with **Roger Nicoll** too and there I studied synapses, how neurons communicate with each other and how these connections can change in the response to transient events that can lead ultimately to memory and learning. Trying to understand what the cellular and molecular basis of memory and learning is. Then after that I went back to Portland, Oregon and this is where I started my work with the auditory system and I did a post-doc there with **Larry Trussell** who was my second post-doctoral mentor and this is how I started applying, if you will to more auditory circuits and I have been working on auditory circuits ever since. Then after completing my post-doc with **Larry Trussell** I started my own lab, initially in Chicago for a couple of years, **Chicago Medical School,** but soon I moved here to the **University of Pittsburgh** where I have been for eleven years and I am studying cellular molecular mechanisms that underline normal and pathological auditory processing, basically how we hear, what is the mechanism of hearing

how, when there are hearing disorders, what can we do when things go wrong. And as I have told you I have been doing that for the last ten/eleven years here and I am an **Endowed Professor of Auditory Physiology** in the **Department of Otolaryngology** and the last couple of years we created **The Pittsburgh Hearing Research Center,** which, again, addresses issues of normal and pathological hearing and it's a combination of investigators with human-related research, animal model-related model research, clinicians. Again, the goal is to understand the underpinnings of hearing and hearing disorders and how we can help. That's briefly my history in terms of research.

07:16 What triggered your interest in tinnitus?

Hazel: What triggered your interest in the auditory system? Was there something specific that got you interested?

Thanos: Now that I am replaying it, when I did my first post-doc I was working on the hippocampus which is the part of the brain where memory is created, memory is formed and when I had finished this post-doc I wanted to go to more of a sensory system where we know what sort of information that comes to that part of the brain, we know what it's good for, a little bit more peripheral if you will. And we know where the inputs are coming from more clearly. Then I got excited about the auditory system because at the time the very first part of the auditory system, the periphery of the auditory system, was thought to be non-plastic if you will, meaning non-modifiable. But I think some of the structures there kind of resemble more central structures that are modifiable and also plastic, so I wanted to study the plasticity mechanism of the auditory system, which was unknown at the time. So that was the initial motivation to go to a sensory system and, most importantly, to the auditory system.

Hazel: How did tinnitus factor in? At what point did that come on to your radar, so to speak?

Thanos: I told you that I started studying the mechanics of memory and learning and that I basically found how transitory experiences can lead to long-lasting perceptions, long-lasting changes if you will such as memory and learning, and as I was starting the auditory system and I was looking at similar mechanisms that happened in the auditory system, at the time tinnitus was the phantom percept, right? It was the sound that we constantly hear and, basically I got interested to understand how this phantom perception is being formed by an initial event or maybe series of events, and I just tried to understand how experience and the plasticity of the brain lead to the establishment of a perception such as tinnitus.

Hazel: I asked that question because not a lot of academics with a neurological background decide to make tinnitus one of their research topics, so it's always interesting to hear how that interest came about and you know, as with any career choice it can be completely by chance or it can be a deliberate choice.

Thanos: Usually a combination of both. In my case my background shaped me. You know I told you I did potassium channels and then synaptic plasticity then auditory system. If you put all of these things together you can sort of predict that something like tinnitus could be a subject of study.

Hazel: And so, at this point would you call yourself a tinnitus researcher, as in do you have a specific interest in tinnitus, or is it more part of this broader endeavour to learn more about neural activity in the auditory pathway?

Thanos: It's hard to put labels but how do I identify myself? Ok, I'm a neuroscientist to start with. I'm trying to understand how the brain works but I have a strong commitment to tinnitus research because really I have been doing tinnitus research for the last ten or twelve years and actually it's a project that has been going on during this whole time. And it's kind of nice and very satisfying to see how to go from a completely unknown mechanism to identifying some mechanism to creating some molecules that might help correct this mechanism and now hopefully going to clinical trials soon. So, I am a neuroscientist, but I am extremely committed and excited to continue with research on tinnitus so you can call me a tinnitus researcher too so that's all good.

11:57 Personal Experience of Tinnitus

Hazel: That's good to hear. Do you have any personal experience with tinnitus? Or anyone you know?

Thanos: Oh, I have tinnitus. However, I didn't have it when I started working on tinnitus. Was I ahead of my time? I don't know. Anyway, I started realising I had tinnitus five years ago but as I told you I started working on tinnitus more than ten years ago. But I do have tinnitus. It's 24/7. I have a high frequency tone. I have some hearing loss too and luckily enough I am not much bothered by it. You know, I can concentrate, I can focus, I can keep going on with my daily activities but there are times where it is almost louder than your voice, so I do have it, yes.

Hazel: Yes, that's pretty loud, but glad to hear that, most of the time at least, you're not bothered by it.

Thanos: Correct, correct.

Hazel: It's interesting that you got it after you started researching tinnitus, because I've spoken to a lot of tinnitus researchers at conferences and such and many of them have told me this, that they developed tinnitus after they had started researching it so, I don't know?

Thanos: It's funny when you said it because when I first started experiencing tinnitus I thought, oh, no, come on I'm telling myself, I'm thinking about tinnitus, I do these papers about tinnitus, I work on tinnitus, I talk to my students all day about tinnitus, maybe I have convinced myself I have it. No, I have it. And then I went and did an audiogram and then there was some hearing loss and the pattern was consistent with the precept.

13:56 Theory/model of how tinnitus is generated

Hazel: Ok, so let's dive a bit more into the research and talk a bit about tinnitus. Maybe start by talking about tinnitus models and then we'll go into the drug that you are developing. So,

can you start by just explaining for our listeners, do you have a theory or model of how tinnitus is generated?

Thanos: Yes, happy to do that. I definitely have a theory, a model as to how tinnitus is happening so it is a phantom percept right so, first of all I would like to talk a little bit about phantom percepts, meaning a percept that is not based on an external stimulus. That's what I mean by phantom percept. So, if we really think about it thinking, and mindfulness, these are all forms of phantom perception, right? Or we can at least say that mindfulness is not possible without imagination. We have to imagine what we are thinking so, even **Aristotle** himself 2500 years ago, in his book on the soul, he said that the soul could not produce thoughts without relying on phantom constructs, so phantom constructs are the core of cognition and thought and mindfulness so phantom constructs are not something bad but something very good but our other question is why do we need to have phantom percepts, these phantom constructs?

So, here's why I think we need them. Our brains, they do these amazing tasks. I believe that what our brains do is they perform an online prediction of reality and I will explain this a little bit more. And this online prediction has to match the speed of reality. It cannot stay behind or faster, so it has to match reality dynamically. And I'll explain a bit better. For example, let's say we're on the street and a car is approaching us and, you know, what the brain is doing is this prediction and it's matching the reality dynamically. If it comes too close to us, we have to jump at the right time to avoid the bad event because if we don't do it at the right time we're not going to exist for much longer. On the other hand, if we jump before the car comes, we'll keep jumping without any reason. I think it's this match of reality that the brain is constantly doing. Ok, how does this link to phantom perception? I guess what I'm trying to say is that the brain never stops. It's not complete silence and then a sound comes and then the brain understands the sound. The brain, in order to perform this task, has to rely on these internally generated representations of basic aspects of the outside world. Evolution has built these things into our brain to help our brain predict and match reality dynamically and with the right timing.

So, I suggest that these internally driven representations of the sound perception such as tones and hissing, and all these other tinnitus precepts, that during tinnitus are kind of released into our consciousness because some sort of desynchronization occurs. This matching of inside and outside state is not happening right. So, how does this desynchronization occur then? I think what happens is that the internal state of the mind, its internal generated percepts, malfunction in concert with the sensory organ. I think what usually happens first is that the sensory organs go bad, either we lose vision or we get older and then the brain, because these sensory organs that basically inform the brain about the external state, and they form the internal state of the mind. All of the mind as I said has its own intrinsic activity. So, I don't know, maybe it was too long with what I think about the brain but what I think is going on is that the brain never stops. The brain keeps hearing, keeps seeing things, without even external stimulus and frankly that's how we live. The brain has to match the external with the internal and it has to do that fast, and accurately, and sometimes that doesn't happen and I think the reason, especially in tinnitus why it doesn't happen, is that what comes to the brain from our organs stops being the right information. As I said we get hearing loss or vision loss or other things, hearing loss and

tinnitus and that can lead to misrepresentation in the brain. So that's my overall hypothesis in understanding of how the brain works and tinnitus.

19:25 Hearing loss and tinnitus

Hazel: But, of course, we know that not everyone with hearing loss develops tinnitus, so do you know what is different about the brain of someone with tinnitus in that regard?

Thanos: Correct, there has to be a damage. This is my hypothesis, fortunately or unfortunately there is not a unifying hypothesis yet about tinnitus. That is ok, that's why we do research and that's what keeps us busy and happy and I am ok with this uncertainty. So, there is a hearing loss, but sometimes the brain might be able to compensate ok about this hearing loss and still create this synchrony, as I told you, between the internal and external world and then we don't have these aberrant, these phantom sounds, when we don't want them. So I think then it's not only auditory networks that participate in the development of tinnitus, there are also non-auditory networks that are getting involved that then determine in the end who is going to get bothered and who is not, who is going to experience it, and to what extent and who is not. What I decided to study is that, especially in the animal models we started simulating noise-induced hearing loss or some noise-induced trauma to mice and then see how the brains are changed with that.

21:05 Interlude

Hazel: Hey there, I hope you're enjoying this podcast so far. I'd like to just take a minute and ask you to reflect on what brings you here. I assume it's because, like me, you have tinnitus. And if you do, I hope it doesn't bother you too much and that you're able to, say, listen to a podcast episode without feeling constantly distracted or aggravated by your tinnitus. But even if you are able to ignore it, remember there are millions of people out there who feel completely debilitated by their tinnitus. And that's got to change. By supporting the Tinnitus Talk Podcast, which you can do through Patreon for as little as 2 dollars a month, you wouldn't just be supporting the podcast itself, but all the other work we do to help people with tinnitus, such as running the Tinnitus Talk support forum, collecting data for research, and working directly with researchers to help push for a cure.

We hope you'll consider it. Either way, we appreciate you listening. And now, back to the interview!

22:13 Potassium channels and influence on nerve activity

Hazel: I know your research also focusses a lot on the molecular level and specifically these potassium channels and how they may change nerve activity. I don't know if I am explaining it well, but can you explain how that factors into the whole model of how tinnitus is generated?

Thanos: When I started working on tinnitus it was known that there are these neurons in the very first stop of the auditory nerve. There is the ear, the sound stimulus in the form of pressure. It enters the ear and then the hair cells get activated from the sound pressure then this pressure is converted to an electrical signal and it goes to our brain and then the brain does its thing. And I'm going to get into that.

So, the very first stop of the auditory nerve to the brain is this area called **cochlear nucleus**. It is the first part of the central auditory nervous system. There was known work before me that was done by **Don Caspary** and also by **Tom Brozoski** and **Kaltenbach**, they showed that these neurons that are in the dorsal cochlear nucleus, they are called fusiform neurons, they fire after noise exposure and after some damage and/or tinnitus. They fire, they have activity when there is not supposed to be activity. They have enhanced spontaneous activity so that early on that's what started thinking that you know maybe this aberrant central activity, maybe this is what triggers tinnitus.

And then there was also some very important work to me ... I think it was the **Brozoski** group that did it, and **Carol Bauer** and **Don Caspary**. I think what they did they ablated the dorsal cochlear nucleus after tinnitus was established in rats and tinnitus was established for three months, then tinnitus was still there. However, when they ablated the structure during the noise exposure, they couldn't induce tinnitus, suggesting that this part of the brain, the cochlear nucleus, is important for the triggering of tinnitus.

So, it is important for the triggering and it leads to this aberrant activity of neurons, these neurons fire. So that's what I knew. So then what I really simply tried to understand is how come these neurons fire more? Why are these neurons more active at a molecular level? I tried to understand why they fire axon potentials, why they are 'talking' more after noise exposure so that's what started the studies. That is what I read when I started, irrelevant of whether that would solve tinnitus or not, just this very basic phenomenon of this hyperactivity of these neurons.

So then, what we did we were able to record from brain slices, basically you can cut very thin slices of this area of the brain and you can look at these neurons in isolation, or can record the electrical activity of these neurons. So, we are able, in vitro, and these brain slices, if you put them in the right solution, they can stay alive for several hours. So, we can look at this activity whilst you record from these neurons. And we were able in this in vitro preparation, we call it 'in vitro' because obviously it is not a live animal, we were able to reproduce this basic effect that the mice that were noise exposed and that had behavioural evidence of tinnitus, they had this hyperactivity.

And then we wanted to see how it is generated and a similar result that we had is that you know the way neurons fire, there are two major ways that neurons will fire. It is because some other neurons will tell them to do it. There is another neuron next to it you know it sends a signal telling it to go ahead and fire. These are the synapses. The activity of the brain is formed by the excitatory and the inhibitory forces. There are neurons that tell you to fire more, there are neurons that tell you to fire less and it's this balance of these excitatory and inhibitory actions that determine the firing of the neurons. So that's one way, the synapses.

The other way is that there are intrinsic activities. By intrinsic activities I mean these neurons, they have these channels, like potassium channels, like sodium channels, like calcium channels that on their own can generate activity on these neurons without any input. And what we initially found is that these neurons are spontaneously active and even if you remove the connections from other neurons you can still see this activity, which was

also increased after tinnitus. So early on we started focussing on what's called intrinsic properties which means we are looking for some channel. One small comment here. I'll stop in case you have questions for the next part, but that doesn't mean that the synapses don't play a role in this activity but because we found that these neurons are so robustly driven by the intrinsic properties we decided to focus on the intrinsic properties.

Ok, let's take a break on this.

28:08 How potassium ion channels might cause tinnitus

Hazel: I think I'm following. So, what do you think is happening in those potassium ion channels that could cause this over-activity in the nerves?

Thanos: Ok, when it comes to intrinsic properties, like channels, there are again breaks and accelerators in the neuron. The sodium channels are the accelerators. They allow for sodium ions to depolarise to make the memory potential more positive and then that leads to the firing of the neuron, the activity of the neuron. As the neuron gets more gets more positive and starts firing then the potassium channel starts to get activated and then they take potassium from the inside of the cell to the outside and then hyper-polarise the quiet down. This is the break of activity. The potassium channel, this is the break of activity. So, simply thinking where we said ok, either we have an added accelerator, or we will have the removal of a break. That's how it would go faster.

To make a long story short, I had a very talented graduate student. Her name is **Shuang Li**. We went through a lot of biophysical and electrophysiological tricks and to make a long story for you, here in a couple of minutes we found out that in a potassium channel one of the breaks was just not working well. Somehow these channels were not operating optimally which means that a break that a neuron had was not existing anymore. OK, the channels don't work, and they do not allow the potassium to come out and that's why there is not that break and why the cells fire which are not supposed to fire.

But the next question was, ok it is a potassium channel. It sounds like all KC and Q channels are KV7 channels. That is less important, but what is important is how do these channels stop working right? What is the problem, why do they not work? Again, there are two major ways that a channel can stop working. One is that these channels they have to synthesise inside the cell, and they have to go to the membrane of the neuron to allow for this in and out of the ions that I told you. It could be that the channels never make it to the membrane, they are not formed somehow after noise trauma and tinnitus. They are ruined for whatever reason.

The other alternative is that the channels are there, but they are not functioning properly, and one major function of channels is that they are voltage gated. It means that depending on the voltage of the membrane they open or close. And think of voltage as the force that you have to apply to open something. Let's say, if it's a heavy door you have to apply more effort, if it's a lighter door, less effort. So, this voltage dependence opens and closes the channels. So what we found is that the channels out of the membrane, if you really apply a lot of force on them you can open them to the same extent as the normal channel, but

somehow the force that you have to apply is larger in order to keep them well functioning. So that was good news, because it means that if we want to do something about it and have these channels open, nature was kind of generous to us. It put them there, but we just had to open them. So how do you open the channel?

Hazel: So, the potassium ion channels, they normally soothe or calm the nerve activity? And your theory is that if the channels are somehow compromised and they don't flow property maybe then they don't perform that soothing function and then you get the overactivity of the nerves in the auditory pathway that cause tinnitus. Is that a correct summary?

Thanos: Excellent. You said it very well. The only thing I would add at this point as the story evolves, we don't know if it causes tinnitus yet but, we can say, exactly as you said beautifully, that this can cause aberrant activity of these neurons. These neurons are active when they are not supposed to be active.

Hazel: And then we assume that that aberrant activity is what causes the tinnitus signal?

Thanos: But is it? So, in order to prove that we have to do something more causal. Can we change that? Can we open these channels now to these damaged mice, to these noise-exposed mice and then can we change the activity of the channel, but also can we prevent them from having tinnitus? So, to me that's the next important step. The good thing is that the structure of these channels, I mean the three-dimensional structures of the channels and how these channels open and close has been known by other researchers. These channels are well characterised, and we know what these channels look like.

So, after having these findings, before I started to collaborate with a chemist to make a new drug, there was this drug out there. This drug was called **Retigabine.** This was **FDA** approved. It's still **FDA** approved. It's just not out there anymore because it's not beneficial to the market. So, this drug was given to people that had epilepsy and it was working. But what does this drug do? This drug does exactly the opposite of what tinnitus did to the channels. I will explain that. This drug is a channel opener. It opens the channels. These KC and Q channels. So how does it do it? Basically, as I told you the channels go through this open and closed state. Imagine that you place a small molecule at a part of the channel that will keep it open. Imagine that you put an obstacle in the door now and this doesn't close, you keep the door open.

So, we figured that, ok, let's try this drug in mice and see do we correct the biophysical activity, the neurons and how the neurons fire, and then do we correct the behaviour. And we did. Both worked. That's why we are talking now. And then when we gave these mice tinnitus, we were able to correct these channels and open them and we were also able to get good behavioural evidence that we were able to correct tinnitus in these mice. So, however, there is a problem with this drug and it's not in the market anymore. First problem is that the channels that I told you about, these KC and Q channels, the KV7 channels, there are five types of these channels, KC and Q1 to KC and Q5. The channels that were identified by us that play a role in tinnitus are the channels KC and Q2 and 3, but this **Retigabine** drug opens KC and Q2, 3, 4 and 5 so already there was a lack of specificity there but also **Retigabine** started having these effects. People that were taking **Retigabine** for a year, again

it was working for epilepsy, they started developing this blue colouration to their skin and their retina due to some degradation products of **Retigabine** and also the lack of specificity of **Retigabine.**

So, the bottom line is if we want to move forward and create a drug that would hopefully help our individuals with tinnitus, we need to create a more specific and more potent and less toxic drug.

36:24 Re-development of existing drug Retigabine

Hazel: And that's what you are trying to do now? You're redeveloping this existing drug to target the problem more specifically and to avoid negative side effects?

Thanos: Correct. You know, science is a collaborative affair and I am by no means a chemist. In order to do that you need a chemist. You need to have somebody who knows the channel well and knows the small molecules well and designs a small molecule now that is very specific and very potent. I was very lucky, and I am grateful that I met **Peter Wipf. Peter Wipf** is a chemist here at **The University of Pittsburgh** and he's a medicinal chemist, not just a chemist. So, we started collaborating on this and with that goal, exactly as you said, how can we now make a more specific and a more potent drug and Peter is a magician when it comes to chemistry. He created several new molecules that were more potent and more specific. They were just binding to these KCV 2 and 3 channels, number one. They don't bind to four and five. They bind with very high affinity, with very high specificity and, also, they are much more stable, and they don't have the degradation products that lead to these toxic effects that **Retigabine** has.

So, now, we are in that stage and we are working towards refining and identifying the lead molecule that we are going to be taking to the clinical trials. I am happy to report that we have made a lot of progress in the pre-clinical development. We have the lead molecule and we have to go through all these regulatory paths, of which there are many, and I am learning them as we are going through, to be able to give it to humans.

The good thing is that, apart from **Peter Wipf**, my department, **The Department of Laryngology,** and **Jon Johnson**, the **Chair,** there is also the **Eye and Ear Foundation** which supports our department. Also, there is the **UPMC, University of Pittsburgh Medical Center,** and also **UPMC Enterprises** that have endorsed, they have been helping us all along with that. Because, you know, the knowledge that you need at that stage is basically I'm a neuroscientist but It takes a lot of knowledge of the market, of regulatory steps, to get a drug to the market so I am very fortunate that we have been endorsed by **UPMC Enterprises** who know these aspects of the game in order to move a drug to the market. Things are advancing slowly and steadily with a high level of commitment.

39:18 Sub-typing of tinnitus

Hazel: That's great to hear and I want to hear more about plans for market launch and the next phases of clinical trial but, maybe first, do you have a sense of whether maybe this drug

would help anyone with tinnitus or is it focussed on specific types of tinnitus? I guess implicit in that question is whether you believe in this concept of sub-typing of tinnitus?

Thanos: I do believe that there are sub-types of tinnitus. However, we do not know what these sub-types are. And, also tinnitus, as we discussed earlier, is a phantom percept. It's very tightly bound to subjectivity. So ok, yes, I do believe there are categories of tinnitus. Of course, we can easily say that there is a noise-induced tinnitus or a drug-induced tinnitus. There are different ways to induce, to get tinnitus, age dependent tinnitus which, maybe they convert somewhere, and that could be peripheral damage. I think our drug would work in cases where there is hyperactivity of the central nervous system. Also, what I believe about our drug, and I cannot predict yet in which category it is going to work, but we are working on it and I will talk to you about that later.

But I think the bottom line is that tinnitus is a plasticity disorder in my mind. There is peripheral damage, or some peripheral change and that leads to central reorganisation to compensate. Sometimes it compensates, sometimes it doesn't but I think plasticity, although it's the cause of the disorder, that it is also the cure of the disorder. All I am trying to say is that if we tweak the system now by using a drug that would quieten these neurons, you'd give it a chance for the system to recalibrate. You'd give it a chance to reach another state that might not be bothersome or that might not include this phantom perception. The bottom line is we need to make sure that this drug is safe to be given to people to start the clinical trials and then as clinical trials evolve we have to become more mindful and hopefully with more knowledge as to what are the best tinnitus sub-categories for which our drug would be most effective, if any.

One more thing, the hope is that for individuals who have epilepsy this drug worked for sure. Of that there is no doubt. Of course, we saw that. But it had all these side-effects which we are trying to correct. So at least part of tinnitus has a resemblance to epilepsy. There is an aberrant activity somewhere in the auditory brain or even in the non-auditory brain and I think that will be corrected and hopefully that correction will help many people who have tinnitus or, at least, bothersome tinnitus. We'll see.

42:37 Potential benefits for hyperacusis

Hazel: Do you think it could also have potential benefits for hyperacusis?

Thanos: It could, it could, although that's another topic but again, in theory, yes. Because if hyperacusis is also a part of increased activity, increased gain of the response it might help there too.

Hazel: Yes, it would seem to make intuitive sense, but I guess we won't know for sure until you start testing on the trials.

43:11 Clinical trials

Hazel: So, let's talk a bit more about the testing, and the clinical trials. So, what stage of the development are you at exactly right now?

Thanos: We are on the refinement of the lead candidate and we do all these in vivo, in vitro, toxicology tests of several compounds that we have, including **RL-81** which is more known because a lot of it has been published, to figure out what is the best lead candidate to move it to the clinic. Now, as I told you, a lot of this is toxicology, pharmacokinetics and, also, we have to 'de-risk' the known risks of **Retigabine.** So, there are extra steps, which is good and bad. The good thing is we know what we are dealing with, we know what the toxicities are, the blue colour, the melanin binding, and the skin discolouration. The bad part, or the challenging part, or the opportunity part, we have a chance to correct these toxic effects and, as I say, we will have a chance to correct it. So that is why the pre-clinical development of this compound has extra steps. Because we know we are dealing with extra toxicity, which is known. But it is moving forward, it is moving forward. So far, so good. There is always go no go test and we have not had a no go test with what is our lead compound for now.

44:51 Animal testing and pre-clinical testing

Hazel: So, you took extra care or time with animal testing, pre-clinical testing. Someone, one of our listeners actually, asked an interesting question that made me curious. Have you conducted any tests that 'failed' and what did you learn from this?

Thanos: During this whole narrative that I gave you, how we reached what is a potassium trial and how does it do it. I mean 90% of the experiments we do are failures. I didn't talk to you about all the failures because we would have to be talking for about, ok, not for ten years, but at least a couple of years. I hope he is listening. Science is full of failures and the ability to deal with failures and learn and move forward so everything I know, and I learn is because of these failures, frankly.

Hazel: That's probably an important message, I think, for the listeners, as well, you know, because people are always wondering: *why does it take so long to develop new treatments*, but they probably don't realize all the trials and tribulations that come with it.

Thanos: I mean, you know, failures are a badge of honor, right? Seriously, in science we're trying to figure out the unknown, and it takes a lot of failures to start believing that something is right until the next experiment. So but, the good thing is that when you see this in a long time—I've been doing that now for 10-11 years—progress has occurred. However at times really when I was doing that stuff, I said forget about it; it's never—I never felt that—with my students and trying channel after channel and drug after drug and neuron after neuron, it felt that it's never happening because 80-90% of the things that we do are unsuccessful because it's hard. It's hard to get to the truth.

Hazel: You have to be very persistent. Yeah.

Thanos: Indeed. Indeed.

Hazel: So you're now at the stage where you want to move from animal testing to human testing, is that correct?

Thanos: Correct. Correct.

Hazel: All right. And so, one thing we've seen with some previous treatments for tinnitus is that the animal models didn't always seem to translate very well to human models and sometimes it led to disappointing results in clinical trials, so how optimistic are you about that?

Thanos: You're right. There are pretty specific changes. Although, a lot of the fundamentals are the same, there are specific changes. To give you some examples for both, these potassium channels KC and Q channels are very very very similar—almost identical—between us and mice. They're very highly conserved, right? It was a successful evolutionary decision that nature made that has been maintaining many species. However now different species are differentially sensitive to sound, right? Some of them can tolerate sound more, some others not, so, I will agree with you that are specific differences and you never know what's going to happen once you go to the human. People are developing. Primate models get closer, if you will, to us, but I think in our case, these potassium channels are very very very homogenous throughout the species, number one. Number two: humans took this drug or a similar drug and it definitely affected neuronal activity. And it helped them, so at least we have these things going for us.

Definitely this channel opener will affect the neuronal properties of human neurons. I'm not doubting that. How that now is going to translate to tinnitus or no tinnitus, frankly remains to be seen. Now I'm optimistic given all these patterns that I described to you. And I'm optimistic that, to say the least, if it doesn't work for tinnitus again, I'm committed to that, it might work for epilepsy, right? Or it might work for pain—or it might work for hyperacusis, okay? So I think—I don't leave this planet until I make sure that this drug is safe enough to be given to humans—that nothing bad is going to happen to them and then I think I'm expecting good things out of this, and is it really going to solve tinnitus for everybody? I do not know that, but I'm optimistic that humans will benefit.

50:09 Timeline for upcoming clinical trials

Hazel: That's good to hear. Can you give us a timeline for the upcoming phases of clinical trials and even the expected market launch?

Thanos: This is tough. Life's expectations are definitely bigger than mine, but okay, I understand why people want to know that. I think that in the next year or two we should be able to 'de-risk' everything that we know and take the drug to clinical trials, so I think in the next year or two, I'm expecting different clinical development to fully be completed and start the clinical trials. After that, frankly, I do not know. There has to be phase 1; there has to be phase 2; there has to be phase 3, but it's all going to depend on multiple factors, but I think for now I can say that I would like to believe that in the next couple of years we will be giving this drug to normal—to individuals without tinnitus or whatever to make sure that it is safe.

Hazel: Yes, because that's always the first phase of human testing—is purely is it safe—not it is effective—but is it safe, right?

Thanos: Correct. And you know we have to go through all of these regulatory aspects of FDA to make sure that in all the animal models—because the preclinical is in animal models—to figure out really we've exhausted all the possibilities that this drug does not have anything toxic in it—because again, you know, keep in mind, we are going to give it to humans and the last thing you want to do is harm them.

Hazel: Yeah, of course. So what are actually the biggest factors that determine the speed at which this can happen?

51:57 Speed of getting the drug to market

Thanos: For us I know, for me and Peter, because we're both scientists and science keeps driving us, right—and, of course, we have the support of the University, and the Foundation, and the UPMC—it was a really—get the right expertise and get—surround ourselves with the right team that will help us make the right decision as to how it is to be moved forward, so this has been a source of delay, at least for us because it was a new thing and we're learning it. And I think that we found a team, and I feel confident that the team knows—they know what they're doing and they're guiding us through each step. So that was a big step because again, keep in mind, with scientists that figured out the basic mechanism and molecule and we're trying to move things to the market, it's so many—there are so many steps that, you know, that we had to learn. So that was a big delay for us, figuring out the right team and the right thing to do. I think we're over that.

Hazel: All right, yeah, as you can imagine a lot of people who are suffering badly from tinnitus are very desperate for a cure sooner rather than later, and there's a lot of frustration around, you know, all the regulation you have to go through—all the steps of the of the FDA, and so a lot of people are asking isn't it possible to apply for a fast track for this drug? Would that be an option?

Thanos: Yeah, I do not know much about that. I have heard that term but I cannot speak about it. I do not know what that is and I don't know if that's an option. I can discuss with my team. Definitely that has not been on the table yet, and especially now with COVID-19 and all that stuff I don't know how appropriate, you know, I'm saying, how much we can push other things, but, bottom line, I don't know the answer and I can ask and learn more and let you know.

Hazel: Yeah, so it sounds like you will need some kind of a commercial partner or who can really help bring this drug to market.

Thanos: But I think the thing that we have now takes care of that, too. They're doing the best job in the development for that. We're in good hands. It is going to happen. Again, this going to go there so I don't have to solve this to be risk all these risks at the I think that we

had and that is an unknown but again we know it needs to get done and I'm optimistic and confident that we're going to figure it out and move forward.

54:33 Partnerships & funding

Hazel: Alright, so when you said that we have sort of everything we need in the team can you reiterate who are actually the different partners?

Thanos: We have people with experience in the team that have taken drugs to the market, people with experience in the team that know how to talk to the FDA and they can guide us what needs to get done in order to get FDA approval to start the—it's called the IND—Investigation for New Drug application—IND—and we have also people that are aware of the market and looking at opportunities out there because even up to phase 1 we can go with what we have, but then things reach phase 2 or phase 3 about the tens of millions, right? And it's a different ball game and then you need some big partners in the team that are aware of this circuitry, if you will, of how to get things done at that level, so that's what I mean by that, and also most importantly, but right now, I'm kind of a more step-by-step type of guy. I know the important thing is to risk the problems of this drug so we can file for an IND and start the phase 1, and I think we have what it takes, but why we're doing this? There are people on the team looking at the next step, how we can go there and get either more money from venture capitalists. I don't know how things are going to evolve, but the experts are following that up and they're helping us.

Hazel: Yeah, yeah, that's good to hear. Let's talk a little bit about funding, though, because my understanding is that you got a grant from the Department of Defense, is that correct?

Thanos: This is the third grant. I've been funded from the Department of Defense for the last 10-11 years and I'm completely grateful and indebted to them. When they first funded me, I figured the first I told you how these neurons are different in brain science and why they fire more, and once they figure out the potassium channels, the DOD funded me again to create a molecule that is better than retic—you know—to create a specific model that's why we started collaborating with **Peter Wipf**, the chemist, so that was another round of funding from DOD. The third round of funding from DOD came a couple of years ago where we said, okay now we have these drug, this compound, and actually family of compounds that work better. Let's identify the best lead compound and then start the clinical development. So this grant does not fund the whole clinical development but then, as I told you, this great support that we get here from the IND Foundation, from the Department of Otolaryngology, but also from UPMC Enterprises—they're helping us further now with a preclinical development right, to reach phase 1. So that has been my funding history, if you will.

Hazel: All right, all right. So you don't think that funding will be an inhibiting factor in going through the clinical trials?

Thanos: Right now, it's not. For reaching a state of completing the preclinical development and going to the clinical—to phase 1—it seems that it doesn't seem to be a factor right now because by this thing that we have, I think we can address that.

Hazel: That's good to hear and let's talk a bit about collaboration, broader collaboration, within tinnitus research fields. Are you following what other tinnitus researchers are doing? Do you go to conferences etc.?

Thanos: Yes, definitely I'm trying to read all the tinnitus-related research and I'm trying to keep up with the other investigators. I go to conferences. The main conference that I go to regularly that I hear at lot of things is the Association for Otolaryngology. It happens every year. I go to that regularly. At times I get invited to other, to different meetings, so yes, to answer your question, it's a fundamental part of what I'm trying to do—stay updated with other researchers and see how we can coordinate and collaborate.

59:06 General impressions of tinnitus research

Hazel: What's your general impression of the tinnitus research field. Is there enough collaboration going on?

Thanos: I guess this would be compared—I'm trying to think now—this question you can answer only by comparing it to some other field. Okay, let me think. I think it's comparable to other fields. It's a new field. I mean still there is not a dominant hypothesis, if you will, or we haven't figured things out yet—the animal model stuff, too, you know, we're talking about subjective perception, which is even if she wants it's hard to tell what you think, what you hear, and what you smell, what I hear, what I smell. So it is a complicated field by definition. It is a new field, but I think everybody's trying to do his or her best, and yeah, I think so—I think that things are well advanced. I don't know. I don't see anything majorly worse or better than other things, you know?

Hazel: All right, right, so no major obstacles, but you're saying it's just still a very young field and there's lots of things to figure out.

Thanos: That's true. That's true.

Hazel: Yeah, have you seen that evolve a lot over the past decade, let's say?

Thanos: Yeah. Yeah, it has. I mean, you know, there are—yes, I'm thinking of work by… our work, **Shore**'s work, **Kilgard**'s work, **Rauschecker**'s work… Yeah, I think things are advancing. You know, we all want it to go faster and better, sure, but I think, yes, things are advancing. There are problems, but as I said, I haven't been in any field that there are not pros and cons and there are no problems, yeah.

Hazel: Yeah, yeah, so here's a kind of closing question that I ask every tinnitus researcher. What can be done by the patient community to advance the cause of research?

Thanos: The cause of research… Hmmm… I think—first, you all have been very active and I want an active and vibrant community and that's great. What more can be done? You know, I haven't thought about it. Um, I don't know. I think it's a newly recognized condition. We're not talking so much about tinnitus 10-15 years ago, and I think that keeps getting better and better. I think you've all been very active and very complementary to our work. You've been asking good questions, you keep us excited, motivated and on our feet to finish what we start. As I told you, I have tinnitus, and I want to see this work completed, right, and hopefully help myself and others. That's why we fight so hard for this cure. That's the objective part—that's why we are trying so hard.

Hazel: Yeah, exactly. Well, I want to thank you on behalf of the tinnitus patient community for all of your hard work, and we're rooting for you to be successful.

Thanos: And thank you, thank you very much for the kind words. Thank you very much for being here. And I want to emphasize I am really committed to continue and finish this line of work—and I'm very committed and excited about it. Again, thank you very much.